

New Journal

Well = Screen.Status.WellName + " " + "Site" + str(Screen.Status.SiteNum)
Power = str(Component.405_Laser_Power.Position) + "% " + "Laser Power"

IF Screen.Status.WaveNum=2 THEN
Segmentation of miRFP for Targeting

EDIT ANALYSIS for MiRFP from here [LINE 1] to [Line 19]

1: New "LowPass" = Basic Filters("Camera NIR", 5, 5)
2: New "Segmentation" = Count Nuclei(Src=[1: Basic Filters])
3: Close([1: Basic Filters])
4: Threshold Image([2: Count Nuclei], 1, 65535, Inclusive)
5: New "miRFP Binary" = Binarize([2: Count Nuclei]), high = current value, low = current value
6: Close([2: Count Nuclei])

IF dilate equals "nucleus", then use nucleus segmentation, but remove objects around the edges. Else, dilate the activation

IF size_dilate = 0 THEN

7: Integrated Morphometry - Load State("exclude-objects-borders")
8: Integrated Morphometry - Measure([5: Binary Operations])
9: Integrated Morphometry - Create Objects Mask()
10: Create Regions Around Objects("IMA Objects Mask")
11: Transfer Regions("IMA Objects Mask", "Camera NIR", ALLREGIONS)
12: Close([5: Binary Operations])
13: Close("IMA Objects Mask")

ELSE

14: Integrated Morphometry - Load State("miRFP Segmentation_Single Bin")
15: Integrated Morphometry - Measure([5: Binary Operations])
16: Set Image Zoom([5: Binary Operations], 100)
17: New "Centroid_Binary" = As Displayed([Last Result], Entire image)
18: Set Color Threshold([17: As Displayed], RGBCOLORMODEL and INCLUSIVEHUE and STATEINCLUSIVE, 254,
19: New "MiRFP Centroid" = Binarize([17: As Displayed]), 1, 255
20: New "Dilate" = Morphological Dilate([19: Binary Operations], Circle)
size = size_dilate
21: Create Regions Around Objects("Dilate")
22: Transfer Regions("Dilate", "Camera NIR", ALLREGIONS)
23: Close([20: Morphological Dilate])
24: Close([19: Binary Operations])
25: Close([17: As Displayed])

END IF

*****TARGETING FOR MOSAIC -NO NEED TO EDIT SCRIPT/CHANGE BELOW THIS LINE*****

26: Select Image("Camera NIR")

IF Image.NumRegions >=1 THEN

Reg = 1
Total_Regions = Image.NumRegions +Total_Regions
Regions are Saved and loaded to Mosaic for Targeting
Run Journal C:\MM\app\mmproc\journals\Mosaic journals\Save ROI Batch 1.jnl
current_illumination = Device.Illumination.Setting
27: Select Illumination("Camera GFP")
28: Targeted Illumination = Targeted Illumination(Illum setting=Camera GFP, Coord setting=20X APO, Active region, I
Coordinate system setting = Device.Magnification.Setting
Mask Exposure Duration [ms] = pulsetime
29: Delay(MILLISEC)
Time = pulsetime
30: Select Illumination("Camera GFP")
Setting Name = current_illumination
31: Clear All Regions("Camera NIR")

ELSE

Reg = 0
Dont' Utilize the Mosaic on this Field of View

```
    END IF  
ELSE  
  
END IF
```

Create Overlay Images for Final Display Purposes

```
IF Screen.Status.WaveName="Camera RFP_532x_Post" THEN
```

```
    IF Reg=1 THEN
```

```
        32: New "Color Combine" = Color Combine("Camera NIR", "Camera GFP", [None])
```

```
        33: Text([32: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 9, "%Well% Pulse Time: %pulsetime% Power: %Power%")
```

```
        34: Load Regions([32: Color Combine], "Batch 1 ROI Targeting")
```

```
        35: Add to "Target Regions Overlay" = As Displayed([32: Color Combine], Entire image)
```

```
        36: Close([32: Color Combine])
```

```
    ELSE
```

```
        37: New "Color Combine" = Color Combine("Camera NIR", "Camera GFP", [None])
```

```
        38: Text([37: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 8, "%Well% Pulse Time: %pulsetime% Power: %Power%")
```

```
        39: Add to "Target Regions Overlay" = As Displayed([37: Color Combine], Entire image)
```

```
        40: Close([37: Color Combine])
```

```
    END IF
```

```
ELSE  
END IF
```