

## 3 Level Segmentation-

Variables and logs that must be pre-opened at the beginning of the experiment:

blank object log (data logging should be paused at beginning of experiment - note that image data will be saved to the datalog; first set of measurements are cytoplasm; second set are nucleus)

IMA for bin 1 pulsetime

IMA for the bin pulsetime2

Pulse1 is Correlated to Blue (High) - 800ms recommended (intensely nuclear) - NEED TO DEFINE, variable = hi\_value

Pulse2 Length is Correlated to Green (Middle) - 200ms recommended (medium nuclear) - NEED TO DEFINE, variable = md\_value

Pulse3 is not used for this experiment

In this experiment, only GFP is imaged (and cropped)

Well = Screen.Status.WellName + "\_" + "Site" + str(Screen.Status.SiteNum)

Power = str(Component.405\_Laser\_Power.Position) + "% " + "Laser Power"

IF Screen.Status.WaveName="Camera GFP" THEN

Crop GFP, maintaining original image name

name = "Camera GFP"+"\_"+"Crop"

1: Create Region()

Position:X/From:X = 0

Position:Y/From:Y = 1

Width/To:X = Image.Width

Height/To:Y = Image.Height-2

2: Image/Plane("Camera GFP")

Image.Name = name

3: Clear All Regions([Current At Start])

Segment cells

4: Run Journal("nuclear-seg\_detail-oriented")

Create bin1 binary (high shape factor)

5: Integrated Morphometry - Load State("high\_shapefactor\_bin")

6: Integrated Morphometry - Measure("Camera GFP\_Crop", mask image = "final\_binary")

(Disabled)7: Integrated Morphometry - Log Data([Last Result], OBJECTS, CURRENTDATA, 1, 2)

8: Integrated Morphometry - Create Objects Mask()

Image.Name = "Reg1\_storage"

9: Create Regions Around Objects([Last Result])

Create bin2 binary (low shape factor)

10: Integrated Morphometry - Load State("low\_shapefactor\_bin")

11: Integrated Morphometry - Measure("Camera GFP\_Crop", mask image = "final\_binary")

(Disabled)12: Integrated Morphometry - Log Data([Last Result], OBJECTS, CURRENTDATA, 1, 2)

13: Integrated Morphometry - Create Objects Mask()

Image.Name = "Reg2\_storage"

14: Create Regions Around Objects([Last Result])

15: Close("final\_binary")

Save regions in Reg1\_storage, then close it

16: Select Image("Reg1\_storage")

IF Image.NumRegions>=1 THEN

Reg1 = 1

FOR Image.ActiveRegion = 1 TO Image.NumRegions STEP 1

Region.ColorBlue = 255

Region.ColorGreen = 0

Region.ColorRed = 0

NEXT

17: Save Regions("Reg1\_storage", "Batch 1 ROI Targeting")

ELSE

```
No Regions Present at the Low Threshold
Reg1 = 0
END IF
18: Close("Reg1_storage")
```

```
Save regions in Reg2_storage, then close it
19: Select Image("Reg2_storage")
IF Image.NumRegions>=1 THEN
    Reg2 = 1
    FOR Image.ActiveRegion = 1 TO Image.NumRegions STEP 1
        Region.ColorBlue = 0
        Region.ColorGreen = 0
        Region.ColorRed = 255
    NEXT
    20: Save Regions("Reg2_storage", "Batch 2 ROI Targeting")
ELSE
    No Regions Present at the Low Threshold
    Reg2 = 0
END IF
21: Close("Reg2_storage")
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```
No 3rd bin so make Reg3 = 0
Reg3 = 0
END Segmentation of Image
22: Close("Camera GFP_crop")
```

\*\*\*\*\*TARGETING FOR MOSAIC -Batch 1 ROI's.....NO NEED TO EDIT SCRIPT/CHANGE BELOW THIS LINE

```
23: Select Image("Camera GFP")
IF Reg1=1 THEN
    Regions are Saved and loaded to Mosaic for Targeting
    24: Load Regions("Camera GFP", "Batch 1 ROI Targeting")
    current_illumination = Device.Illumination.Setting
    25: Select Illumination("Camera GFP")
    26: 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
    27: Delay(MILLISEC)
        Time = pulsetime
    28: Select Illumination("Camera GFP")
        Setting Name = current_illumination
    29: Clear All Regions("Camera GFP")
ELSE
    Reg1 = 0
    Dont' Utilize the Mosaic on this Field of Vlew
END IF
```

\*\*\*\*\*TARGETING FOR MOSAIC -Batch 2 ROI's.....NO NEED TO EDIT SCRIPT/CHANGE BELOW THIS LINE

```
30: Select Image("Camera GFP")
IF Reg2=1 THEN
    Regions are Saved and loaded to Mosaic for Targeting
    31: Load Regions("Camera GFP", "Batch 2 ROI Targeting")
    current_illumination = Device.Illumination.Setting
    32: Select Illumination("Camera GFP")
    33: 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] = pulsetime2
```

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34: Delay(MILLISEC)
    Time = pulsetime2
35: Select Illumination("Camera GFP")
    Setting Name = current_Illumination
36: Clear All Regions("Camera GFP")
ELSE
    Dont' Utilize the Mosaic on this Field of View
END IF

```

\*\*\*\*\*TARGETING FOR MOSAIC -Batch 3 ROI's.....NO NEED TO EDIT SCRIPT/CHANGE BELOW THIS LINE

```

37: Select Image("Camera GFP")
IF Reg3=1 THEN
    Regions are Saved and loaded to Mosaic for Targeting
38: Load Regions("Camera GFP", "Batch 3 ROI Targeting")
    current_illumination = Device.Illumination.Setting
39: Select Illumination("Camera GFP")
40: 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] = pulsetime3
41: Delay(MILLISEC)
    Time = pulsetime3
42: Select Illumination("Camera GFP")
    Setting Name = current_Illumination
43: Clear All Regions("Camera GFP")
ELSE
    Reg1 = 0
    Dont' Utilize the Mosaic on this Field of View
END IF
44: Select Image("Camera GFP")

```

ELSE

END IF

Create Overlay Images for Final Display Purposes: Conditions: (1,1,1) (1,1,0) (1,0,1) (1,0,0) (0,1,1) (0,1,0) (0,0,1) (0,0,0)

```

IF Screen.Status.WaveName="Camera GFP" THEN
    IF Reg1=1 THEN
        IF Reg2=1 THEN
            IF Reg3=1 THEN
                Lo, Mid and High Regions Found Reg1=1 and Reg 2=1 and Reg3=1
                Reg1=1 and Reg2=1 and Reg3=1
                #1 (1,1,1)
                (Disabled)45: New "Color Combine" = Color Combine([None], "Camera GFP", [None])
                (Disabled)46: Text([Nonexistent command], 10, 10, 0, 255, 0, Arial, Bold, 18, "%Well% Pulse Time Hi: %pu
                (Disabled)47: Load Regions([Nonexistent command], "Batch 1 ROI Targeting")
                (Disabled)48: Load Regions([Nonexistent command], "Batch 2 ROI Targeting")
                (Disabled)49: Load Regions([Nonexistent command], "Batch 3 ROI Targeting")
                (Disabled)50: Add to "Target Regions Overlay" = As Displayed([Nonexistent command], Entire image)
                (Disabled)51: Close([Nonexistent command])
            ELSE
                #2(1,1,0)
                (Disabled)52: New "Color Combine" = Color Combine([None], "Camera GFP", [None])
                (Disabled)53: Text([Nonexistent command], 10, 10, 0, 255, 0, Arial, Bold, 18, "%Well% Pulse Time Hi: %pu
                (Disabled)54: Load Regions([Nonexistent command], "Batch 1 ROI Targeting")
                (Disabled)55: Load Regions([Nonexistent command], "Batch 2 ROI Targeting")
            END IF
        END IF
    END IF

```

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        (Disabled)56: Add to "Target Regions Overlay" = As Displayed([Nonexistent command], Entire image)
        (Disabled)57: Close([Nonexistent command])
    END IF
ELSE
    Reg1=1 and Reg 2=0 Reg3=1
    IF Reg3=1 THEN
        #3 (1,0,1)
        (Disabled)58: New "Color Combine" = Color Combine([None], "Camera GFP", [None])
        (Disabled)59: Text([Nonexistent command], 10, 10, 0, 255, 0, Arial, Bold, 18, "%Well%    Pulse Time Hi: %pu
        (Disabled)60: Load Regions([Nonexistent command], "Batch 1 ROI Targeting")
        (Disabled)61: Load Regions([Nonexistent command], "Batch 3 ROI Targeting")
        (Disabled)62: Add to "Target Regions Overlay" = As Displayed([Nonexistent command], Entire image)
        (Disabled)63: Close([Nonexistent command])
    ELSE
        #4 (1,0,0)
        (Disabled)64: New "Color Combine" = Color Combine([None], "Camera GFP", [None])
        (Disabled)65: Text([Nonexistent command], 10, 10, 0, 255, 0, Arial, Bold, 18, "%Well%    Pulse Time Hi: %pu
        (Disabled)66: Load Regions([Nonexistent command], "Batch 1 ROI Targeting")
        (Disabled)67: Add to "Target Regions Overlay" = As Displayed([Nonexistent command], Entire image)
        (Disabled)68: Close([Nonexistent command])
    END IF
END IF
ELSE
    Reg1=0 and Reg2=1
    IF Reg2=1 THEN
        Reg1=0 and Reg2=1 and Reg3=1
        IF Reg3=1 THEN
            #5 (0,1,1)
            (Disabled)69: New "Color Combine" = Color Combine([None], "Camera GFP", [None])
            (Disabled)70: Load Regions([Nonexistent command], "Batch 2 ROI Targeting")
            (Disabled)71: Load Regions([Nonexistent command], "Batch 3 ROI Targeting")
            (Disabled)72: Text([Nonexistent command], 10, 10, 0, 255, 0, Arial, Bold, 18, "%Well%    Pulse Time Hi: %pu
            (Disabled)73: Add to "Target Regions Overlay" = As Displayed([Nonexistent command], Entire image)
            (Disabled)74: Close([Nonexistent command])
        ELSE
            #6 (0,1,0)
            (Disabled)75: New "Color Combine" = Color Combine([None], "Camera GFP", [None])
            (Disabled)76: Load Regions([Nonexistent command], "Batch 2 ROI Targeting")
            (Disabled)77: Text([Nonexistent command], 10, 10, 0, 255, 0, Arial, Bold, 18, "%Well%    Pulse Time Hi: %pu
            (Disabled)78: Add to "Target Regions Overlay" = As Displayed([Nonexistent command], Entire image)
            (Disabled)79: Close([Nonexistent command])
        END IF
    ELSE
        IF Reg3=1 THEN
            #7 (0,0,1)
            (Disabled)80: New "Color Combine" = Color Combine([None], "Camera GFP", [None])
            (Disabled)81: Load Regions([Nonexistent command], "Batch 3 ROI Targeting")
            (Disabled)82: Text([Nonexistent command], 10, 10, 0, 255, 0, Arial, Bold, 18, "%Well%    Pulse Time Hi: %pu
            (Disabled)83: Add to "Target Regions Overlay" = As Displayed([Nonexistent command], Entire image)
            (Disabled)84: Close([Nonexistent command])
        ELSE
            #8 (0,0,0)
            (Disabled)85: New "Color Combine" = Color Combine([None], "Camera GFP", [None])
            (Disabled)86: Text([Nonexistent command], 10, 10, 0, 255, 0, Arial, Bold, 18, "%Well%    Pulse Time Hi: %pu
            (Disabled)87: Add to "Target Regions Overlay" = As Displayed([Nonexistent command], Entire image)
            (Disabled)88: Close([Nonexistent command])
        END IF
    END IF
END IF
ELSE

```

END IF