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3 Level Segmentation-

Low Expression MiRFP in Blue, Middle in Green and High in Red-

Pulse1 is Correlated to Blue (Low) - 800ms recommended
Pulse2 Length is Correlated to Green (Middle) - 200ms recommended
Pulse3 Length is Correlated  to Red (High) - 50ms recommended

Note that GFP/Dendra2 image must be taken after miRFP image. So, images should go GFP --> NIR --> RFP (post)

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
    Reg1 = 0
    Reg2 = 0
    Reg3 = 0
    Segmentation of miRFP for Targeting: Threshold level after segmentation to creat binay imageLow Gray (0-1) Middle Gree
    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])
    Segment Bottom Quartile
    7: Integrated Morphometry - Load State("0-Q1")
    8: Integrated Morphometry - Measure("Camera NIR", mask image = [5: Binary Operations])
    9: Integrated Morphometry - Log Data("Camera NIR", OBJECTS, CURRENTDATA, 1, 2)
    10: Integrated Morphometry - Create Objects Mask()
    11: Create Regions Around Objects("IMA Objects Mask")
    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
        12: Save Regions("IMA Objects Mask", "Batch 1 ROI Targeting")
    ELSE
        No Regions Present at the Low Threshold
    END IF
    13: Close("IMA Objects Mask")

    Segment Second Quartile Nuclei
    14: Integrated Morphometry - Load State("Q1-Q2")
    15: Integrated Morphometry - Measure("Camera NIR", mask image = [5: Binary Operations])
    16: Integrated Morphometry - Log Data("Camera NIR", OBJECTS, CURRENTDATA, 1, 2)
    17: Integrated Morphometry - Create Objects Mask()
    18: Create Regions Around Objects("IMA Objects Mask")
    IF Image.NumRegions>=1 THEN
        Reg2 = 1
        FOR Image.ActiveRegion = 1 TO Image.NumRegions STEP 1
            Region.ColorBlue = 0
            Region.ColorGreen = 255
            Region.ColorRed = 0
        NEXT
        19: Save Regions("IMA Objects Mask", "Batch 2 ROI Targeting")
    ELSE
        No Regions Present at the Low Threshold
    END IF
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20: Close("IMA Objects Mask")

Segment Third Quartile Nuclei
21: Integrated Morphometry - Load State("Q2-Q3")
22: Integrated Morphometry - Measure("Camera NIR", mask image = [5: Binary Operations])
23: Integrated Morphometry - Log Data("Camera NIR", OBJECTS, CURRENTDATA, 1, 2)
24: Integrated Morphometry - Create Objects Mask()
25: Create Regions Around Objects("IMA Objects Mask")
IF Image.NumRegions>=1 THEN
    Reg3 = 1
    FOR Image.ActiveRegion = 1 TO Image.NumRegions STEP 1
        Region.ColorBlue = 0
        Region.ColorGreen = 0
        Region.ColorRed = 255
    NEXT
    26: Save Regions("IMA Objects Mask", "Batch 3 ROI Targeting")
ELSE
    No Regions Present at the Low Threshold
END IF
27: Close("IMA Objects Mask")
28: Close([5: Binary Operations])
END Segmentation of Image




**********TARGETING FOR MOSAIC -Batch 1 ROI's.....NO NEED TO EDIT SCRIPT/CHANGE BELOW THIS LINE
29: Select Image("Camera NIR")
IF Reg1=1 THEN
    Regions are Saved and loaded to Mosaic for Targeting
    30: Load Regions("Camera NIR", "Batch 1 ROI Targeting")
    current_illumination = Device.Illumination.Setting
    31: Select Illumination("Camera GFP")
    32: 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
    33: Delay(MILLISEC)
        Time = pulsetime
    34: Select Illumination("Camera GFP")
        Setting Name = current_Illumination
    35: Clear All Regions("Camera NIR")
ELSE
    Reg1 = 0
    Dont' Utilize the Mosaic on this Field of VIew
END IF
36: Select Image("Camera NIR")




**********TARGETING FOR MOSAIC -Batch 2 ROI's.....NO NEED TO EDIT SCRIPT/CHANGE BELOW THIS LINE
IF Reg2=1 THEN
    Regions are Saved and loaded to Mosaic for Targeting
    37: Load Regions("Camera NIR", "Batch 2 ROI Targeting")
    current_illumination = Device.Illumination.Setting
    38: Select Illumination("Camera GFP")
    39: Targeted Illumination = Targeted Illumination(Illum setting=Camera GFP, Coord setting=20X APO, Active region, I
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            Coordinate system setting = Device.Magnification.Setting
            Mask Exposure Duration [ms] = pulsetime2
        40: Delay(MILLISEC)
            Time = pulsetime2
        41: Select Illumination("Camera GFP")
            Setting Name = current_Illumination
        42: Clear All Regions("Camera NIR")
    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
    43: Select Image("Camera NIR")
    IF Reg3=1 THEN
        Regions are Saved and loaded to Mosaic for Targeting
        44: Load Regions("Camera NIR", "Batch 3 ROI Targeting")
        current_illumination = Device.Illumination.Setting
        45: Select Illumination("Camera GFP")
        46: 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
        47: Delay(MILLISEC)
            Time = pulsetime3
        48: Select Illumination("Camera GFP")
            Setting Name = current_Illumination
        49: Clear All Regions("Camera NIR")
    ELSE
        Reg1 = 0
        Dont' Utilize the Mosaic on this Field of VIew
    END IF
    50: Select Image("Camera NIR")


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 RFP_532x_Post" 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)
                51: New "Color Combine" = Color Combine("Camera NIR", "Camera GFP", [None])
                52: Text([51: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 11, "%Well%     Pulse Time Hi: %pulsetime%     Pu
                53: Load Regions([51: Color Combine], "Batch 1 ROI Targeting")
                54: Load Regions([51: Color Combine], "Batch 2 ROI Targeting")
                55: Load Regions([51: Color Combine], "Batch 3 ROI Targeting")
                56: Add to "Target Regions Overlay" = As Displayed([51: Color Combine], Entire image)
                57: Close([51: Color Combine])
            ELSE
                #2(1,1,0)
                58: New "Color Combine" = Color Combine("Camera NIR", "Camera GFP", [None])
                59: Text([58: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 11, "%Well%     Pulse Time Hi: %pulsetime%     Pu
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            60: Load Regions([58: Color Combine], "Batch 1 ROI Targeting")
            61: Load Regions([58: Color Combine], "Batch 2 ROI Targeting")
            62: Add to "Target Regions Overlay" = As Displayed([58: Color Combine], Entire image)
            63: Close([58: Color Combine])
        END IF
    ELSE
        Reg1=1 and Reg 2=0 Reg3=1
        IF Reg3=1 THEN
            #3 (1,0,1)
            64: New "Color Combine" = Color Combine("Camera NIR", "Camera GFP", [None])
            65: Text([64: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 11, "%Well%    Pulse Time Hi: %pulsetime%    Pu
            66: Load Regions([64: Color Combine], "Batch 1 ROI Targeting")
            67: Load Regions([64: Color Combine], "Batch 3 ROI Targeting")
            68: Add to "Target Regions Overlay" = As Displayed([64: Color Combine], Entire image)
            69: Close([64: Color Combine])
        ELSE
            #4 (1,0,0)
            70: New "Color Combine" = Color Combine("Camera NIR", "Camera GFP", [None])
            71: Text([70: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 11, "%Well%    Pulse Time Hi: %pulsetime%    Pu
            72: Load Regions([70: Color Combine], "Batch 1 ROI Targeting")
            73: Add to "Target Regions Overlay" = As Displayed([70: Color Combine], Entire image)
            74: Close([70: Color Combine])
        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)
            75: New "Color Combine" = Color Combine("Camera NIR", "Camera GFP", [None])
            76: Load Regions([75: Color Combine], "Batch 2 ROI Targeting")
            77: Load Regions([75: Color Combine], "Batch 3 ROI Targeting")
            78: Text([75: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 11, "%Well%    Pulse Time Hi: %pulsetime%    Pu
            79: Add to "Target Regions Overlay" = As Displayed([75: Color Combine], Entire image)
            80: Close([75: Color Combine])
        ELSE
            #6 (0,1,0)
            81: New "Color Combine" = Color Combine("Camera NIR", "Camera GFP", [None])
            82: Load Regions([81: Color Combine], "Batch 2 ROI Targeting")
            83: Text([81: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 11, "%Well%    Pulse Time Hi: %pulsetime%    Pu
            84: Add to "Target Regions Overlay" = As Displayed([81: Color Combine], Entire image)
            85: Close([81: Color Combine])
        END IF
    ELSE
        IF Reg3=1 THEN
            #7 (0,0,1)
            86: New "Color Combine" = Color Combine("Camera NIR", "Camera GFP", [None])
            87: Load Regions([86: Color Combine], "Batch 3 ROI Targeting")
            88: Text([86: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 11, "%Well%    Pulse Time Hi: %pulsetime%    Pu
            89: Add to "Target Regions Overlay" = As Displayed([86: Color Combine], Entire image)
            90: Close([86: Color Combine])
        ELSE
            #8 (0,0,0)
            91: New "Color Combine" = Color Combine("Camera NIR", "Camera GFP", [None])
            92: Text([91: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 11, "%Well%    Pulse Time Hi: %pulsetime%    Pu
            93: Add to "Target Regions Overlay" = As Displayed([91: Color Combine], Entire image)
            94: Close([91: Color Combine])
        END IF
    END IF
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    END IF
ELSE
END IF
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