```
New Journal

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

IF Screen.Status.WaveNum=1 THEN
    Segmentation of miRFP for Targeting

    EDIT ANALYSIS for MiRFP from here [LINE 1] to [Line 19]
    1: New "LowPass" = Basic Filters("Camera GFP", 5, 5)
    2: New "Segmentation" = Count Nuclei(Src=[1: Basic Filters])
    3: Close([1: Basic Filters])
    4: Threshold Image([2: Count Nuclei], 1, 255, Inclusive)
    5: New "miRFP Binary" = Binarize([2: Count Nuclei]), high = current value, low = current value
    6: Close([2: Count Nuclei])
    IF size_dilate = 0 THEN
        7: Create Regions Around Objects([5: Binary Operations])
        8: Transfer Regions([5: Binary Operations], "Camera GFP", ALLREGIONS)
        9: Close([5: Binary Operations])
    ELSE
        10: Integrated Morphometry - Load State("miRFP Segmentation_Single Bin")
        11: Integrated Morphometry - Measure([5: Binary Operations])
        12: Set Image Zoom([5: Binary Operations], 100)
        13: New "Centroid_Binary" = As Displayed([Last Result], Entire image)
        14: Set Color Threshold([13: As Displayed], RGBCOLORMODEL and INCLUSIVEHUE and STATEINCLUSIVE, 254,
        15: New "MiRFP Centroid" = Binarize([13: As Displayed]), 1, 255
        16: New "Dilate" = Morphological Dilate([15: Binary Operations], Circle)
            size = size_dilate
        17: Create Regions Around Objects("Dilate")
        18: Transfer Regions("Dilate", "Camera GFP", ALLREGIONS)
        19: Close([16: Morphological Dilate])
        20: Close([15: Binary Operations])
        21: Close([5: Binary Operations])
        22: Close([13: As Displayed])
    END IF


    Make the ROi's the same Color for overlay purposes later (Example Hi Intensity vs. Low Signal)
    23: Select Image("Camera GFP")
    IF Image.NumRegions>=1 THEN
        FOR Image.ActiveRegion = 1 TO Image.NumRegions STEP 1
            Region.ColorBlue = 255
            Region.ColorGreen = 0
            Region.ColorRed = 0
        NEXT
    ELSE
        No Regions Present
    END IF

    **********TARGETING FOR MOSAIC -NO NEED TO EDIT SCRIPT/CHANGE BELOW THIS LINE**********
    24: Select Image("Camera GFP")
    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
        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
        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
        30: New "Color Combine" = Color Combine("Camera RFP_532x_Post", "Camera GFP", [None])
        31: Text([30: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 12, "%Well% Pulse Time: %pulsetime%  Power: %Power%
        32: Load Regions([30: Color Combine], "Batch 1 ROI Targeting")
        33: Add to "Target Regions Overlay" = As Displayed([30: Color Combine], Entire image)
        34: Close([30: Color Combine])
    ELSE
        35: New "Color Combine" = Color Combine("Camera RFP_532x_Post", "Camera GFP", [None])
        36: Text([35: Color Combine], 10, 10, 0, 255, 0, Arial, Bold, 12, "%Well% Pulse Time: %pulsetime%  Power: %Power%
        37: Add to "Target Regions Overlay" = As Displayed([35: Color Combine], Entire image)
        38: Close([35: Color Combine])
    END IF
ELSE
END IF
```