**SUPPLEMENTARY SOFTWARE CODE**

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Supplemental Code 42. set image zoom and scaling.JNL

Supplemental Code 43. Stitch Stack and Save Images.JNL

Note: Custom variables are highlighted in red and MetaMorph variables are highlighted in green.

**Supplemental Code 1. Preliminary Scan.JNL**

1: "5x TMA Prelim Scan" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)
2: Show Message and Wait("Select the 5X objective. If calibration has not already been run, do so now, Confirm channel and exposure. Then set left upper and right lower positions to define the scan area", USE TIMEOUT)

\*\*\*End of Journal\*\*\*

**Supplemental Code 2. Save Prelim Scan Settings.JNL**

1: "5x TMA Prelim Scan" = Scan Slide Save Settings()

\*\*\*End of Journal\*\*\*

**Supplemental Code 3. Run Scan.JNL**

1: Scan Slide

\*\*\*End of Journal\*\*\*

**Supplemental Code 4. segment TMA.JNL**

1: StartImage = Image.Name

2: Overwrite "LowPass" = Basic Filters([Current At Start], 10, 10)

3: Overwrite "Adaptive Threshold" = Adaptive Threshold([2: Basic Filters], 20, 2000, 100)

4: Close([2: Basic Filters])

5: Overwrite "Multiply" = [Last Result] \* 1 / 1

6: Threshold Image([Last Result], 1, 65535, Inclusive)

7: Select Image("Adaptive Threshold result")

slide = Prompt User(Which slide is this (1 - 4)?)

row = Prompt User(How many rows does the TMA have (1 - 10)?)

column = Prompt User(How many columns does the TMA have (1 - 10)?)

FirstROINumber = Prompt User(What is the number of the first ROI on this grid?)

8: *Run Journal* For Each Grid Position([3: Adaptive Threshold], 5, 8, 0, 96, 96, 28, 28, "transfer shrink transfer TMA")

Number of Columns = column

Number of Rows = row

Horizontal Spacing = 96

Vertical Spacing = 96

Anchor Column = 1

Anchor Row = 1

9: *Run Journal*("Clean up grid")

\*\*\*End of Journal\*\*\*

**Supplemental Code 5. transfer shrink transfer TMA.JNL**

1: Transfer Regions([Current At Start], "Multiply", SELECTEDREGION and CLEARDEST)

2: Select Image("16 bit Adaptive Threshold result")

3: RegionRow = if(len(Region.Label) = 3,left(Region.Label,1),left(Region.Label,2))
4: RegionColumn = Right(Region.Label, 1)
5: Region.Label = STR(((VAL(slide) - 1) \* VAL(row) \* VAL(column)) + (((VAL(RegionRow) - 1) \* 5) + VAL(RegionColumn)))

6: Shrink Region to Fit("Multiply")

7: Transfer Regions("Multiply", "%StartImage%", SELECTEDREGION and CLEARSOURCE)

\*\*\*End of Journal\*\*\*

**Supplemental Code 6. Clean up grid.JNL**

1: Close("Multiply")
2: Close("Adaptive Threshold")

\*\*\*End of Journal\*\*\*

**Supplemental Code 7. Rename Region Label.JNL**

1: Region.Label = Enter Variable("Enter New Region Number")

\*\*\*End of Journal\*\*\*

**Supplemental Code 8. Reload One ROI Set.JNL**

1: Show Message and Wait("Is the window into which the ROIs will be loaded selected as the current active window? ", NO TIMEOUT)
ROIsetLoad = Prompt User(String)
2: Load Regions([Current At Start])

Region File = ScanSlide.Directory + "\Regions-Slide " + STR(ROIsetLoad) + ".RGN"

\*\*\*End of Journal\*\*\*

**Supplemental Code 9. AddToAllROINumbers.JNL**

1: Show Message and Wait("Is the window with the ROIs the current active window? WARNING: All currently loaded ROIs will be renumbered.", NO TIMEOUT)
AugmentRegionNumber = Prompt User(Enter the value to add to each ROI number.)
2: *Loop for all Regions*([Current At Start], "IncreaseROINumbers")

\*\*\*End of Journal\*\*\*

**Supplemental Code 10. IncreaseROINumbers.JNL**

1: Region.Label = str(val(Region.Label) + val(AugmentRegionNumber))

\*\*\*End of Journal\*\*\*

**Supplemental Code 11. Save and clear ROIs.JNL**

ROIsetNumber = Prompt(Which ROI set (slide) is this (1 - 4)?)
1: "5x TMA Prelim Scan" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)
2: Save Regions([Current At Start])

Region File = ScanSlide.Directory + "\Regions-Slide " + STR(ROIsetNumber) + ".RGN"
3: Clear All Regions([Current At Start])

\*\*\*End of Journal\*\*\*

**Supplemental Code 12. TMA Scan (open).JNL**

1: "TMA scan current settings" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)
2: Show Message and Wait("Select the objective to be used for final imaging. Confirm that wavelengths, focus offsets, shading correction, saving location, and journal settings are correct.", USE TIMEOUT)

\*\*\*End of Journal\*\*\*

**Supplemental Code 13. Save TMA Scan Settings.JNL**

1: "TMA scan current settings" = Scan Slide Save Settings()

\*\*\*End of Journal\*\*\*

**Supplemental Code 14. Reload ROIs.JNL**

ROISetsTotal = Prompt(How many sets of ROIs (slides) will be loaded (1 - 4)?)
1: "TMA scan current settings" = Scan Slide Save Settings()
2: "5x TMA Prelim Scan" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

3: ROISetCount = 1

WHILE ROISetCount < (VAL(ROISetsTotal )+1) DO

4: Load Regions([Current At Start])
Region File = ScanSlide.Directory + "\Regions-Slide " + STR(ROIsetCount) + ".RGN"

5: ROISetCount = ROISetCount + 1

WEND

6: Show Message and Wait("Use the Scan Slide tools to position the 20X objective over several regions across all slides. For each region, use Move All Regions within the Regions tools to adjust positioning if needed. Continue when adjustments have caused all regions to be correctly placed.", USE TIMEOUT)
7: "TMA scan current settings" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

\*\*\*End of Journal\*\*\*

**Supplemental Code 15. Save all slide ROIs.JNL**

1: ROIgroup = Enter Variable("Which ROI group should be saved? ")

IF ROIgroup = "adjusted" THEN

2: Show Message and Wait("All ROIs displayed will be saved as "RegionsAllslides(adjusted).RGN" in the scan slide directory.", USE TIMEOUT)

3: "5x TMA Prelim Scan" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

4: Save Regions([Current At Start])

Region File = ScanSlide.Directory + "\Regions-All Slides(adjusted).RGN"

5: "TMA scan current settings" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

ELSE

END IF

IF ROIgroup = "original" THEN

6: Show Message and Wait("All ROIs displayed will be saved as "Regions-Allslides.RGN" in the scan slide directory. ", USE TIMEOUT)

7: "5x TMA Prelim Scan" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

8: Save Regions([Current At Start])

Region File = ScanSlide.Directory + "\Regions-All Slides.RGN"

9: "TMA scan current settings" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

ELSE

END IF

IF ROIgroup = "rescan" THEN

10: Show Message and Wait("All ROIs displayed will be saved as "Rescan Regions.RGN" in the rescan directory. ", USE TIMEOUT)

11: "TMA rescan current settings" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

12: Save Regions([Current At Start])

Region File = ScanSlide.Directory + "\Rescan Regions.RGN"

ELSE

END IF

\*\*\*End of Journal\*\*\*

**Supplemental Code 16. DuplicatePrelimROIs.JNL**

1: New "Untitled" = As Displayed([Current At Start], Entire image)

2: Image.FilePath = ScanSlide.Directory + "\PrelimScanWithROIs.tif"
3: Save([Last Result])
4: Close([Last Result])

\*\*\*End of Journal\*\*\*

**Supplemental Code 17. FocusAcquireSettings.JNL**

1: FocusChannel = Enter Variable("Which channel's focus acquire settings are you checking (choose one)?")

2: AcquireSettingFile = Journal.RunJournalLastDirectory + "acquisitions\autofocusing" + FocusChannel + ".AST"

3: "autofocusing…." = Acquire - Load Setting(EXPOSURE and BINNING and REGION and ILLUMINATION and DISPLAY and SETTING NAME and SAVING and CORRECTION and CORRIMAGES and ANNOTATION and SPECIAL and COLOR SCALING and LIVE REPLAY)

File Name = AcquireSettingFile

4: Show Message and Wait("Press Acquire Press Acquire. Determine threshold (next button). Repeat in and out of focus over areas with and without tissue. Repeat for each channel used.", USE TIMEOUT)

\*\*\*End of Journal\*\*\*

**Supplemental Code 18. Threshold finder.JNL**

1: Scale Image([Current At Start], AUTOSCALE, 50, 20)
2: Difference = Image.ScaleValueHigh - Image.ScaleValueLow

3: Show Message and Wait("Threshold = %Difference% 20th percentile = %Image.ScaleValueHigh% 50th percentile = %Image.ScaleValueLow%; Be sure to test away from the plane of focus. This value is used to determine if tissue is present. ", NO TIMEOUT)

\*\*\*End of Journal\*\*\*

**Supplemental Code 19. Save Focus Settings.JNL**

*Lines 1 and 2 can be enabled in in a future MM release to automate saving according to the wavelength selected in step 1. To do this, override the save file setting in line 2 and specify File Name = AcquireSettingFile.*

*1: FocusChannel = Enter Variable("Which channel's focus acquire settings are you saving (choose one)?")
2: AcquireSettingFile = Journal.RunJournalLastDirectory + "acquisitions\autofocusing" + FocusChannel + ".AST"*

3: "autofocusing…" = Acquire - Save Setting()

\*\*\*End of Journal\*\*\*

**Supplemental Code 20. OpenPrelimAndROIs.JNL**

1: Show Message and Wait("The preliminary scan must be in the save directory specified in Preliminary 5X scan. If not, press cancel.", NO TIMEOUT)

2: "5x TMA Prelim Scan" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

3: "Preliminary 5X scan" = Open Scan()

Scan File Name = ScanSlide.Directory + "\Preliminary 5X scan.scan"

4: *Run Journal*("LoadAdjustedRegions")

\*\*\*End of Journal\*\*\*

**Supplemental Code 21. LoadAdjustedRegions.JNL**

1: ROIgroup = Enter Variable("Which ROI group should be loaded? ")

2: ROIDirectory = ScanSlide.Directory

IF ROIgroup = "original" THEN

3: Show Message and Wait("The original ROI group must be in the save directory specified in Preliminary 5X scan. If not, press cancel. ", NO TIMEOUT)

4: Load Regions([Current At Start])

Region File = ROIDirectory + "\Regions-All Slides.RGN"

5: "TMA scan current settings" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

ELSE

END IF

IF ROIgroup = "adjusted" THEN

6: Show Message and Wait("The adjusted ROI group must be in the save directory specified in Preliminary 5X scan. If not, press cancel. ", NO TIMEOUT)

7: Load Regions([Current At Start])

Region File = ROIDirectory + "\Regions-All Slides(adjusted).RGN"

8: "TMA scan current settings" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

ELSE

END IF

IF ROIgroup = "rescan" THEN

9: Show Message and Wait("The rescan ROI group must be in the save directory specified in TMA rescan current settings. If they are not press cancel.", NO TIMEOUT)

10: "TMA rescan current settings" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

11: ROIDirectory = ScanSlide.Directory

12: Load Regions([Current At Start])

Region File = ROIDirectory + "\Rescan Regions.RGN"

ELSE

END IF

\*\*\*End of Journal\*\*\*

**Supplemental Code 22. SaveCurrentAndUsedSettings.JNL**

1: "TMA scan current settings" = Scan Slide Save Settings()

2: ScanSlideSettingFile = ScanSlide.Directory
3: "TMA scan - settings used" = Scan Slide Save Settings()

\*\*\*End of Journal\*\*\*

**Supplemental Code 23. TMA Scanning Master.JNL**

1: *Run Journal*("SlideLayoutSettings")

2: *Run Journal*("SlideROIFocusSettings")

3: *Run Journal*("DpFnFocusSettings")

SkipConfigurationQuestions = Prompt User(YesNo)

IF SkipConfigurationQuestions = "Y" THEN

4: SSFocus = "Y"

5: Mag = "20x"

6: SlideLayout = "Y"

7: SlideFocSettings = "Y"

ELSE

8: Homed = Enter Variable("Has the stage been calibrated?")

9: Mag = Enter Variable("Choose the objective used for collecting the hi res image")

10: Focus = Enter Variable("Is the %Mag% objective in place and has Z been set to a central focal point for all slides/ROIs? ")

11: AcquireSettings = Enter Variable("Have acquire settings files been created for each channel used for autofocusing (e.g., autofocusingEx\_Em.AST) and saved in the acquisitions subdirectory? ")

12: ScanSettings = Enter Variable("Have the correct TMA Scan Settings been saved and loaded in the Scan Slide window? ")

IF (homed = "N") OR (focus = "N") OR (AcquireSettings = "N") OR (ScanSettings = "N") THEN

Exit Playback

ELSE

END IF

13: SlideLayout = Enter Variable("Are you using the default slide layout? (%TotalSlideNumber% slides; First/Focus ROIs: Slide1 = %FirstROISlide1%, %FocusROISlide1%; Slide2 = %FirstROISlide2%, %FocusROISlide2%; Slide3 = %FirstROISlide3%, %FocusROISlide3%; Slide4 = %FirstROISlide4%, %FocusROISlide4%.")

IF SlideLayout = "N" THEN

14: TotalSlideNumber = Enter Variable("How many slides will be imaged (1 - 4)? (All slides in the open image file that have ROIs will be imaged.) ")

15: FirstROIPos1 = Enter Variable("Is the first ROI position on the first slide numbered 1?")

IF (FirstROIPos1 = "Y") THEN

16: FirstROISlide1 = 1

ELSE

17: FirstROISlide1 = Enter Variable("What number is the first ROI of the first slide?")

END IF

18: FocusROISlide1 = Enter Variable("Which ROI should be used to determine a central focal point for the first slide?")

IF TotalSlideNumber > 1 THEN

19: FirstROISlide2 = Enter Variable("What number is the first ROI of the second slide?")

20: FocusROISlide2 = Enter Variable("Which ROI should be used to determine a central focal point for the second slide? ")

ELSE

21: FirstROISlide2 = 1000

22: FocusROISlide2 = 1000

END IF

IF TotalSlideNumber > 2 THEN

23: FirstROISlide3 = Enter Variable("What number is the first ROI of the third slide?")

24: FocusROISlide3 = Enter Variable("Which ROI should be used to determine a central focal point for the third slide? ")

ELSE

25: FirstROISlide3 = 1000

26: FocusROISlide3 = 1000

END IF

IF TotalSlideNumber > 3 THEN

27: FirstROISlide4 = Enter Variable("What number is the first ROI of the fourth slide?")

28: FocusROISlide4 = Enter Variable("Which ROI should be used to determine a central focal point for the fourth slide? ")

ELSE

29: FirstROISlide4 = 1000

30: FocusROISlide4 = 1000

END IF

ELSE

END IF

31: SlideFocSettings = Enter Variable("Are you using the default slide focus settings? Focus channel = %TotalZFocCh%; Thresholds = %TotalZThreshold%, %RepeatTotalZThreshold%; Z Range = %TotalZRange% um; Step Size = %TotalZFocStepSz% um; Acceptable Focus Variation = %TotalZFocVar% um. ")

IF SlideFocSettings = "N" THEN

32: TotalZFocCh = Enter Variable("Which excitation-emission wavelength will be used to determine the central focal plane of each slide (select one.)? Label should be present in all tissue areas. ")

33: TotalZThreshold = Enter Variable("What scaling difference in this channel should indicate that tissue is present (50 - 2,000)? Use the "threshold finder" to determine this. ")

34: RepeatTotalZThreshold = Enter Variable("What reduced scaling difference in this channel should be used to detect tissue at tissue (50 - 2,000)? ")

35: TotalZRange = Enter Variable("What is the total z range across all slides (100 - 1,000 um)? This is the range that will be checked when finding the central focal plane for each slide; it must include the entire Z range across all slides (centered on the current Z). 500 um is often a reasonable setting. ")

36: TotalZFocStepSz = Enter Variable("How large should steps be when defining the central focal plane of each slide (5 - 20 um)? This will be used to determine the number of steps, e.g., if the Z-step size is 10 um and a focal range of 450 um is entered, 46 steps will be needed. ")

37: TotalZFocVar = Enter Variable("What is the maximum acceptable difference between two identified focus points when determining the central focal plane for each slide (3 - 15 um)? ")

ELSE

END IF

38: ROIFocSettings = Enter Variable("Are you using the default ROI focus settings? Focus channel = %ROIZFocCh%; Threshold = %ROIZThreshold%; ROI Z Range = %ROIZFocRange% um; Step Size = %ROIZFocStepSz% um; Acceptable Focus Variation = %ROIZFocVar% um. ")

IF ROIFocSettings = "N" THEN

39: ROIZFocCh = Enter Variable("Which excitation-emission wavelengths will be used to determine the central focal plane of each ROI (select one.)? Label should be present in all tissue areas. ")

IF ROIZFocCh = TotalZFocCh THEN

40: ROIZThreshold = TotalZThreshold

ELSE

41: ROIZThreshold = Enter Variable("What scaling difference in this channel should indicate that tissue is present (50 - 2,000)? Use the "threshold finder" to determine this. ")

END IF

42: ROIZFocRange = Enter Variable("What is the maximum z range within any one slide (10 - 150 um)? This is the range that will be checked when determining the z origin for each ROI (around the central Z for each slide). 75 um is often a reasonable setting. ")

43: ROIZFocStepSz = Enter Variable("How large should steps be when determining the z origin within each ROI? This will be used to determine the number of steps, e.g., if the Z-step size is 2 um and a focal range of 20 um is entered, 11 steps will be needed. ")

44: ROIZFocVar = Enter Variable("How much difference between two focal points is acceptable when defining the z origin within a single ROI (2 - 5 um)? ")

ELSE

END IF

45: SSfocus = Enter Variable("When imaging tiles within ROIs, do you want focus at defined intervals (instead of built-in Scan Slide focus function; this saves time)? ")

IF (SSfocus = "Y") THEN

46: ScanslideCheck = Enter Variable("Have you set the "scan slide adjust" journal to run at the start of each stage position in Scan Slide? ")

ELSE

IF ScanslideCheck = "N" THEN

Exit Playback

ELSE

47: ScanSlideFocSettings = Enter Variable("Are you using the default settings for deep and fine focusing within ROIs? Deep Focus: Interval = %DpFocInt%; Channel = %DpFocCh%; Thresholds = %DpThreshold%, %ReducedDpFocThreshold%; Range = %DpFocRange% um; Step Size = %DpStepSz% um; Acceptable Focus Variation = %DpFocVar% um. Fine Focus: Interval = %FnFocInt%; Channel = %FnFocCh%; Threshold = %FnThreshold%; Range = %FnFocRange% um; Step Size = %FnFocStepSz% um; Acceptable Focus Variation = %FnFocVar% um. ")

IF ScanSlideFocSettings = "N" THEN

48: DpFocInt = Enter Variable("How often should a deep focus check be made within a ROI (2 - 50)? Set this to a multiple of the fine focus interval. that will be entered. ")

49: ROIZFocCh = Enter Variable("Which excitation-emission wavelengths will be used to determine the central focal plane of each ROI (select one.)? Label should be present in all tissue areas, ")

50: DpFocCh = Enter Variable("Which excitation-emission wavelengths should be used for deep focusing within a ROI? Select only one. ")

51: DpThreshold = Enter Variable("What scaling difference in this channel should indicate that tissue is present (50 - 2,000)? Use the "threshold finder" to determine this. ")

52: ReducedDpFocThreshold = Enter Variable("If reduced thresholds are needed, what value should be used when makine deep focus checks (50 -2000)? ")

53: DpFocRange = Enter Variable("When making deep focus checks within a ROI, how many microns should be scanned (5 -20 microns)? ")

54: DpStepSz = Enter Variable("How large should the steps be when performing a deep focus check (1 - 5 microns)? ")

55: DpFocVar = Enter Variable("How much difference between a new deep focus point and the previous deep focus point within a ROI is acceptable (1 - 5 um)? ")

56: FnFocInt = Enter Variable("How many tiles should be imaged before checking the fine focus? 5 tiles is reasonable. ")

57: FnFocRange = Enter Variable("When making fine focus checks in a ROI, how many microns should be scanned (0.5 - 10 um; this should be equal to or greater than the acceptable deep focus variation)? ")

58: FnFocCh = Enter Variable("When scanning ROIs, which excitation-emission wavelengths should be used for fine focusing? Select only one.

59: FnThreshold = Enter Variable("What scaling difference in the %FnFocCh% channel should indicate that tissue is present (50 - 2,000)? Use the "threshold finder" to determine this. ")

60: FnFocStepSz = Enter Variable("How large should the steps be when checking the fine focus (0.1 - 1 um)?")

61: FnFocVar = Enter Variable("How much difference between a new fine focus point and the previous fine focus point is acceptable (1 - 4 um)? ")

ELSE

END IF

END IF

END IF

END IF

62: InitialZ = Device.Focus.CurPos

63: SlideZ = InitialZ

64: Slide1Z = InitialZ

65: Slide2Z = InitialZ

66: Slide3Z = InitialZ

67: Slide4Z = InitialZ

68: SlideROIZ1 = InitialZ

69: Reset Stopwatch(1)

70: time = New(2, 2, 1, 0)

71: StartTime = Image.Time.Create.String

72: Start Stopwatches(1)

73: Close("time")

74: Close Edge List Log()

75: tempdir = ScanSlide.Directory + "\settings\_and\_report.LOG"

76: Open Edge List Log(OPENFILE and OVERWRITEMODE, "settings\_and\_report")

File Path = tempdir

77: Annotate Log File(EDGELIST, "TMA Scanning Summary")

78: Annotate Log File(EDGELIST, ".................................................................................")

79: Annotate Log File(EDGELIST, "These data are saved in the file:")

80: Log Variable(tempdir, NEWLINE, NO HEADER)

81: Annotate Log File(EDGELIST, "")

82: Annotate Log File(EDGELIST, ".................................................................................")

83: Annotate Log File(EDGELIST, "ROI Focusing Erorrs:")

84: Annotate Log File(EDGELIST, "(If blank there were no focusing errors)")

Position = 1

85: NewArray = Enter Variable("Do you need to create a new stage list array (select "no" if using existing stack)?")

IF NewArray = "N" THEN

86: ActiveImage = Enter Variable("Is "Stage list array.tif" open and selected as the active image?")

IF ActiveImage = "Y" THEN

87: TotalPlanes = Image.NumPlanes

88: *Run Journal*("Region label to array (existing stage list array)")

ELSE

Exit Playback

END IF

ELSE

89: Show Message and Wait("If creating a new Stage list array.TIF file, is the preliminary scan with adjusted ROIs loaded as the active window? If not, press Cancel to exit this process.", NO TIMEOUT)

90: Load Memory List("EMPTY LIST")

91: Scan Slide Regions To Stage List

92: Move To Selected Position(LISTORDER, "add top left or bottom right position to array")

Position = 1

93: *Loop for all Regions*([Current At Start], "Region label to array")

94: Select Image("Choose stage list array image")

95: Image.FilePath = ScanSlide.Directory + "\Stage list array.tif"

96: Save("Stage list array")

END IF

97: *Loop for all Planes*("stage list array", "scan tissue")

98: Close("STAGE LIST ARRAY")

99: Pause Stopwatches(1)

100: EndTime = Image.Time.Create.String

101: *Run Journal*("Record variables")

102: Reset Stopwatch(1)

103: View Current Edge List Log()

104: Show Message and Wait("The log can be reopened using the "Review

TMA Scan Report" button on the taskbar.", USE TIMEOUT)

\*\*\*End of Journal\*\*\*

**Supplemental Code 24. SlideLayoutSettings.JNL**

1: TotalSlideNumber = 4
2: FirstROISlide1 = 1
3: FocusROISlide1 = 23
4: FirstROISlide2 = 41
5: FocusROISlide2 = 62
6: FirstROISlide3 = 81
7: FocusROISlide3 = 103
8: FirstROISlide4 = 121
9: FocusROISlide4 = 137

\*\*\*End of Journal\*\*\*

**Supplemental Code 25. SlideROIFocusSettings.JNL**

1: TotalZRange = 200
2: TotalZFocCh = "360\_440"
3: TotalZThreshold = 500
4: RepeatTotalZThreshold = 100
5: TotalZFocStepSz = 15
6: TotalZFocVar = 5
7: ROIZFocRange = 100
8: ROIZFocCh = "360\_440"
9: ROIZThreshold = 400
10: ReducedROIZThreshold = 100
11: ROIZFocStepSz = 10
12: ROIZFocVar = 5

\*\*\*End of Journal\*\*\*

**Supplemental Code 26. DpFnFocusSettings.JNL**

1: DpFocCh = "360\_440"
2: DpFocRange = 15
3: DpThreshold = 500
4: ReducedDpThreshold = 120
5: DpStepSz = 2
6: DpFocVar = 4
7: DpFocInt = 20
8: FnFocCh = "488\_527"
9: FnFocRange = 4.5
10: FnThreshold = 700
11: ReducedFnThreshold = 100
12: FnFocStepSz = .4
13: FnFocVar = 2.5
14: FnFocInt = 5

\*\*\*End of Journal\*\*\*

**Supplemental Code 27. add top left or bottom right position to array.JNL**

IF ImageExists("stage position") = 0 THEN

1: stage position = New(2, 2, 1, 0)

ELSE
END IF

IF (Position / 2) <> Floor(Position / 2) THEN

2: Select Image("choose stage position "image"")
3: Image.StageX = Device.Stage.XPosition
4: Image.StageY = Device.Stage.YPosition
5: Add Plane("stage position", "stage list array", NOCLOSESOURCE)

ELSE

6: Select Image("choose stage list array")
7: Image.ActivePlane = image.numplanes
8: Image.CameraX = Device.Stage.XPosition
9: Image.CameraY = Device.Stage.YPosition

END IF

10: Position = Position + 1

\*\*\*End of Journal\*\*\*

**Supplemental Code 28. Region label to array (existing stage list array).JNL**

1: Position = 1
2: TotalPlanes = Image.NumPlanes

WHILE Position < (TotalPlanes + 1) DO

3: Image.ActivePlane = Position
4: Label = val(Image.StageLabel)

5: Select Image("choose stage list array")
6: Image.ActivePlane = Position

7: Image.Annotation = ScanSlide.Directory
8: Image.IllumSetting = "Scan " + Device.Magnification.Setting + " ROI " + Str(Label)
9: TopLeftX = Image.StageX
10: TopLeftY = Image.StageY
11: BottomRightX = Image.CameraX
12: BottomRightY = Image.CameraY
IF (Label = FocusROISlide1) OR (Label = FocusROISlide2) OR (Label = FocusROISlide3) OR (Label = FocusROISlide4)

*Run Journal* C:\MM\app\mmproc\journals\Current TMA Scan Slide Scanning Journals\SlideZ focus.JNL

ELSE
END IF

13: Position = Position + 1

WEND

\*\*\*End of Journal\*\*\*

**Supplemental Code 29. Region label to array.JNL**

1: Label = val(Region.Label)

2: Select Image("choose stage list array")
3: Image.ActivePlane = Position
4: Image.StageLabel = Str(Label)

5: Image.Annotation = ScanSlide.Directory
6: Image.IllumSetting = "Scan " + Mag + " ROI " + Str(Label)
7: TopLeftX = Image.StageX
8: TopLeftY = Image.StageY
9: BottomRightX = Image.CameraX
10: BottomRightY = Image.CameraY
IF (Label = FocusROISlide1) OR (Label = FocusROISlide2) OR (Label = FocusROISlide3) OR (Label = FocusROISlide4) THEN

*Run Journal* C:\MM\app\mmproc\journals\Current TMA Scan Slide Scanning Journals\SlideZ focus.JNL

ELSE
END IF

11: Position = Position + 1

\*\*\*End of Journal\*\*\*

**Supplemental Code 30. SlideZ focus.JNL**

1: FocusChannel = TotalZFocCh

*Run Journal* C:\MM\app\mmproc\journals\Current TMA Scan Slide Scanning Journals\Load Autofocus Acquire Settings.JNL

2: CurrentSlide = IF(Label < FirstROISlide2, 1, IF(Label < FirstROISlide3, 2, IF(Label < FirstROISlide4, 3, 4)))

3: Device.Stage.XPosition = (TopLeftX + BottomRightX) / 2
4: Device.Stage.YPosition = (TopLeftY + BottomRightY) / 2
5: Device.Focus.CurPos = InitialZ

6: SlideROIZcount = 0

7: SlideROIZ1 = InitialZ
8: SlideROIZ2 = InitialZ
9: SlideROIZ3 = InitialZ

10: CurrentX = Device.Stage.XPosition
11: CurrentY = Device.Stage.YPosition

12: Select Image("choose stage list array")
13: XRange = abs(TopLeftX - BottomRightX)
14: YRange = abs(TopLeftY - BottomRightY)

15: moveXY = 4
16: Width = XRange / moveXY
17: Height = YRange / moveXY

18: Pattern = "110112212110100101"

WHILE SlideROIZcount <3 DO

19: HowFarToMoveX = (val(left(Pattern,1)) - 1) \* (Width)
20: HowFarToMoveY = (val(mid(Pattern,2,1)) - 1) \* (Height)

21: Device.Stage.XPosition = CurrentX + HowFarToMoveX

22: Device.Stage.YPosition = CurrentY + HowFarToMoveY

23: Device.Focus.CurPos = InitialZ

24: Acquire()
25: Scale Image([Last Result], AUTOSCALE, 50, 20)

TotalZThreshold
26: TissuePresent = IF(Image.ScaleValueHigh - Image.ScaleValueLow > TotalZThreshold, 1, 0)
27: SlideZFocExp = Image.Annotation
28: Close([Last Result])

IF TissuePresent = 1 THEN

29: Adjust Focus(2, 2.5, BACKLASH)

Number of Steps = TotalZRange/ TotalZFocStepSz
Step Size = TotalZFocStepSz

30: Adjust Focus(2, 2.5, BACKLASH)

Number of Steps = TotalZRange/ (TotalZFocStepSz \* 3)
Step Size = TotalZFocStepSz / 3

IF SlideROIZcount = 0 THEN

31: SlideROIZ1 = Device.Focus.CurPos
32: SlideROIZcount = SlideROIZcount+1

ELSE

IF SlideROIZcount=1 THEN

33: SlideROIZ2 = Device.Focus.CurPos
34: SlideROIZcount = SlideROIZcount + 1

IF ABS(SlideROIZ1 - SlideROIZ2) < (TotalZFocVar) THEN

35: SlideZ = (SlideROIZ1+ SlideROIZ2)/2
36: SlideROIZcount = 4

ELSE
END IF

ELSE

37: SlideROIZ3 = Device.Focus.CurPos

IF ABS(SlideROIZ1 - SlideROIZ3) < (TotalZFocVar) THEN

SlideZSlideZ

38: SlideZ = (SlideROIZ1+SlideROIZ3)/2
39: SlideROIZcount = 4

ELSE

IF ABS(SlideROIZ2 - SlideROIZ3) < (TotalZFocVar) THEN

40: SlideZ = (SlideZ2+ SlideZ3)/2
41: SlideROIZcount = 4

ELSE

42: SlideROIZcount = 0

END IF

END IF

END IF

END IF

ELSE
END IF

43: Pattern = mid(Pattern,3)

IF LEN(Pattern)=0 THEN
IF SlideROIZcount < 4 THEN

44: *Run Journal*("SlideZ-TissueNotFound")

ELSE
END IF

ELSE
END IF

WEND

45: Slide1Z = IF(CurrentSlide = 1, SlideZ, Slide1Z)
46: Slide2Z = IF(CurrentSlide = 2, SlideZ, Slide2Z)
47: Slide3Z = IF(CurrentSlide = 3, SlideZ, Slide3Z)
48: Slide4Z = IF(CurrentSlide = 4, SlideZ, Slide4Z)
49: Device.Focus.CurPos = InitialZ

\*\*\*End of Journal\*\*\*

**Supplemental Code 31. SlideZ-TissueNotFound.JNL**

1: Device.Stage.XPosition = (TopLeftX + BottomRightX) / 2

2: Device.Stage.YPosition = (TopLeftY + BottomRightY) / 2

3: Device.Focus.CurPos = InitialZ

4: RepeatSlideROIZcount = 0

5: SlideROIZ1 = InitialZ

6: SlideROIZ2 = InitialZ

7: SlideROIZ3 = InitialZ

8: Pattern = "110112212110100101"

WHILE RepeatSlideROIZcount <3 DO

9: HowFarToMoveX = (val(left(Pattern,1)) - 1) \* (Width)

10: HowFarToMoveY = (val(mid(Pattern,2,1)) - 1) \* (Height)

11: Device.Stage.XPosition = CurrentX + HowFarToMoveX

12: Device.Stage.YPosition = CurrentY + HowFarToMoveY

13: Device.Focus.CurPos = InitialZ

14: Acquire()

15: Scale Image([Last Result], AUTOSCALE, 50, 10)

16: TissuePresent = IF(Image.ScaleValueHigh - Image.ScaleValueLow > RepeatTotalZThreshold, 1, 0)

17: SlideZFocExp = Image.Annotation

18: Close([Last Result])

IF TissuePresent = 1 THEN

19: Adjust Focus(11, 0.4, BACKLASH)

Number of Steps = TotalZRange/ TotalZFocStepSz

Step Size = TotalZFocStepSz

20: Adjust Focus(11, 0.4, BACKLASH)

Number of Steps = TotalZRange/ (TotalZFocStepSz \* 3)

Step Size = TotalZFocStepSz / 3

IF RepeatSlideROIZcount = 0 THEN

21: SlideROIZ1 = Device.Focus.CurPos

22: RepeatSlideROIZcount = RepeatSlideROIZcount +1

ELSE

IF RepeatSlideROIZcount=1 THEN

23: SlideROIZ2 = Device.Focus.CurPos

24: RepeatSlideROIZcount = RepeatSlideROIZcount + 1

IF ABS(SlideROIZ1 - SlideROIZ2) < (TotalZFocVar) THEN

25: SlideZ = (SlideROIZ1+ SlideROIZ2)/2

26: RepeatSlideROIZcount = 4

ELSE

END IF

ELSE

27: SlideROIZ3 = Device.Focus.CurPos

IF ABS(SlideROIZ1 - SlideROIZ3) < (TotalZFocVar) THEN

28: SlideZ = (SlideROIZ1+SlideROIZ3)/2

29: RepeatSlideROIZcount = 4

ELSE

IF ABS(SlideROIZ2 - SlideROIZ3) < (TotalZFocVar) THEN

30: SlideZ = (SlideZ2+ SlideZ3)/2

31: RepeatSlideROIZcount = 4

ELSE

32: RepeatSlideROIZcount = 0

END IF

END IF

END IF

END IF

ELSE

END IF

33: Pattern = mid(Pattern,3)

IF LEN(Pattern)=0 THEN

34: message = "Consensus focus could not be found for the designated ROI in Slide" + CurrentSlide

35: Log Variable(message, NEWLINE, NO HEADER)

36: SlideZ = SlideROIZ1

37: RepeatSlideROIZcount = 4

ELSE

END IF

WEND

38: SlideROIZcount = 4

39: Slide1Z = IF(CurrentSlide = 1, SlideZ, Slide1Z)

40: Slide2Z = IF(CurrentSlide = 2, SlideZ, Slide2Z)

41: Slide3Z = IF(CurrentSlide = 3, SlideZ, Slide3Z)

42: Slide4Z = IF(CurrentSlide = 4, SlideZ, Slide4Z)

43: Device.Focus.CurPos = InitialZ

\*\*\*End of Journal\*\*\*

**Supplemental Code 32. Load Autofocus Acquire Settings.JNL**

1: SuitePath = Journal.RunJournalLastDirectory
2: AcquireSettingFile = Journal.RunJournalLastDirectory + "acquisitions\autofocusing" + FocusChannel + ".AST"
3: "autofocusing…" = Acquire - Load Setting("autofocusing….." = Acquire - Load Setting(EXPOSURE and BINNING and REGION and ILLUMINATION and DISPLAY and SETTING NAME and CORRECTION and CORRIMAGES and ANNOTATION and SPECIALS and COLOR SCALING)

File Name = AcquireSettingFile

\*\*\*End of Journal\*\*\*

**Supplemental Code 33. Scan tissue.JNL**

1: ReviseThreshold = 0

2: ScanSlide.BaseName = str(Image.IllumSetting)
3: Name = str(Image.StageLabel)

Directory = ScanSlide.Directory
4: CurrentSlide = IF(Label < FirstROISlide2, 1, IF(Label < FirstROISlide3, 2, IF(Label < FirstROISlide4, 3, 4)))

5: ScanSlide.ScanUpperLeft.X = Image.StageX
6: ScanSlide.ScanUpperLeft.Y = Image.StageY
7: ScanSlide.ScanLowerRight.X = Image.CameraX
8: ScanSlide.ScanLowerRight.Y = Image.CameraY

*Run Journal* C:\MM\app\mmproc\journals\Current TMA Scan Slide Scanning Journals\ROI focus.JNL

9: DpFocChk = DpFocInt + 1
10: FnFocChk = FnFocInt + 1
11: FirstSubZ = 1

12: Scan Slide

\*\*\*End of Journal\*\*\*

**Supplemental Code 34. ROI focus.JNL**

1: FocusChannel = ROIZFocCh
2: Label = Val(Image.StageLabel)
3: ErrorCode = 3

*Run Journal* C:\MM\app\mmproc\journals\Current TMA Scan Slide Scanning Journals\Load Autofocus Acquire Settings.JNL

4: SlideZ = IF(CurrentSlide = 1, Slide1Z, IF(CurrentSlide = 2, val(Slide2Z), IF(CurrentSlide = 3, val(Slide3Z), IF(CurrentSlide = 4, val(Slide4Z),InitialZ))))

5: Device.Stage.XPosition = (ScanSlide.ScanUpperLeft.X + ScanSlide.ScanLowerRight.X ) / 2
6: Device.Stage.YPosition = (ScanSlide.ScanUpperLeft.Y + ScanSlide.ScanLowerRight.Y ) / 2

7: Device.Focus.CurPos = SlideZ

8: CurrentX = Device.Stage.XPosition
9: CurrentY = Device.Stage.YPosition

10: Select Image("choose stage list array")
11: XRange = abs(Image.StageX - Image.CameraX)
12: YRange = abs(Image.StageY - Image.CameraY)

13: moveXY = 4
14: Width = XRange / moveXY
15: Height = YRange / moveXY

16: Pattern = "110112212110100101"

17: ROIZ1 = SlideZ
18: ROIZ2 = SlideZ
19: ROIZ3 = SlideZ
20: ROIZcount = 0

WHILE ROIZcount <3 DO

21: HowFarToMoveX = (val(left(Pattern,1)) - 1) \* (Width)
22: HowFarToMoveY = (val(mid(Pattern,2,1)) - 1) \* (Height)
23: Device.Stage.XPosition = CurrentX + HowFarToMoveX
24: Device.Stage.YPosition = CurrentY + HowFarToMoveY

25: Device.Focus.CurPos = SlideZ
26: Acquire()
27: ROIZFocExp = Image.Annotation

28: Scale Image([Last Result], AUTOSCALE, 50, 20)
ROIZThreshold

29: TissuePresent = IF(Image.ScaleValueHigh - Image.ScaleValueLow > ROIZThreshold, 1, 0)
30: Close([26: Acquire])

IF TissuePresent = 1 THEN

31: Adjust Focus(11, 0.4, BACKLASH)

Number of Steps = ROIZFocRange/ROIZFocStepSz
Step Size = ROIZFocStepSz

32: Adjust Focus(11, 0.4, BACKLASH )

Number of Steps = ROIZFocRange / (ROIZFocStepSz \* 5)
Step Size = ROIZFocStepSz / 4

IF ROIZcount = 0 THEN

33: ROIZ1 = Device.Focus.CurPos
34: ROIZ = ROIZ1
35: ROIZcount = ROIZcount+1

ELSE

IF ROIZcount=1 THEN
36: ROIZ2 = Device.Focus.CurPos
37: ROIZcount = ROIZcount+1

IF ABS(ROIZ1 - ROIZ2) < ROIZFocVar THEN

38: ROIZ = (ROIZ1+ ROIZ2)/2

39: ROIZcount = 4

ELSE

40: ROIZcount = 1

END IF
ELSE

41: ROIZ3 = Device.Focus.CurPos
IF ABS(ROIZ1 - ROIZ3) < ROIZFocVar THEN

42: ROIZ = (ROIZ1+ ROIZ3)/2

43: ROIZcount = 4
ELSE

44: ROIZcount = 1

IF ABS(ROIZ2 - ROIZ3) < ROIZFocVar THEN

45: ROIZ = (ROIZ2+ ROIZ3)/2

46: ROIZcount = 4
ELSE

47: ROIZcount = 0

 END IF
 END IF
 END IF
END IF
ELSE
END IF

48: Pattern = mid(Pattern,3)

IF LEN(Pattern)=0 THEN

IF ROIZcount < 4 THEN

49: *Run Journal*("ROITissueNotFound")
ELSE

 END IF
 ELSE
 END IF
WEND
50: Device.Focus.CurPos = ROIZ

\*\*\*End of Journal\*\*\*

**Supplemental Code 35. ROITissueNotFound.JNL**

1: ErrorCode = 6
2: ReviseThreshold = 1
*Run Journal* C:\MM\app\mmproc\journals\Current TMA Scan Slide Scanning Journals\Load Autofocus Acquire Settings.JNL

3: Device.Stage.XPosition = (ScanSlide.ScanUpperLeft.X + ScanSlide.ScanLowerRight.X ) / 2
4: Device.Stage.YPosition = (ScanSlide.ScanUpperLeft.Y + ScanSlide.ScanLowerRight.Y ) / 2

5: Device.Focus.CurPos = SlideZ

6: CurrentX = Device.Stage.XPosition
7: CurrentY = Device.Stage.YPosition

8: PatternAdjust = "110112212110100101"

9: ROIZ = SlideZ
10: ROIZ1 = SlideZ
11: ROIZ2 = SlideZ
12: ROIZ3 = SlideZ
13: ReducedROIZcount = 0

WHILE ReducedROIZcount <3 DO

14: HowFarToMoveX = (val(left(PatternAdjust,1)) - 1) \* (Width)
15: HowFarToMoveY = (val(mid(PatternAdjust,2,1)) - 1) \* (Height)
16: Device.Stage.XPosition = CurrentX + HowFarToMoveX
17: Device.Stage.YPosition = CurrentY + HowFarToMoveY

18: Device.Focus.CurPos = SlideZ
19: Acquire()
20: Scale Image([19: Acquire], AUTOSCALE, 50, 10)
21: TissuePresent = IF(Image.ScaleValueHigh - Image.ScaleValueLow > ReducedROIZThreshold, 1, 0)
22: Close([19: Acquire])

IF TissuePresent = 1 THEN

23: Adjust Focus(2, 2.5, BACKLASH)

Number of Steps = 3 \* ROIZFocRange/ROIZFocStepSz
Step Size = ROIZFocStepSz /2

24: Adjust Focus(2, 2.5, BACKLASH)

Number of Steps = ROIZFocRange / (ROIZFocStepSz \* 2)
Step Size = ROIZFocStepSz / 4

IF ReducedROIZcount = 0 THEN

25: ROIZ1 = Device.Focus.CurPos
26: ROIZ = ROIZ1
27: ReducedROIZcount = ReducedROIZcount+1

ELSE

IF ReducedROIZcount=1 THEN

28: ROIZ2 = Device.Focus.CurPos
29: ReducedROIZcount = ReducedROIZcount+1

IF ABS(ROIZ1 - ROIZ2) < ROIZFocVar THEN

30: ROIZ = (ROIZ1+ ROIZ2)/2
 31: ReducedROIZcount = 4

ELSE
END IF

ELSE

32: ROIZ3 = Device.Focus.CurPos
IF ABS(ROIZ1 - ROIZ3) < ROIZFocVar THEN

33: ROIZ = (ROIZ1+ ROIZ3)/2
34: ReducedROIZcount = 4

ELSE

IF ABS(ROIZ2 - ROIZ3) < ROIZFocVar THEN

35: ROIZ = (ROIZ2+ ROIZ3)/2
36: ReducedROIZcount = 4

ELSE
END IF

END IF

END IF

END IF
ELSE
END IF

37: ErrorCode = ReducedROIZcount
38: PatternAdjust = mid(PatternAdjust,3)
IF LEN(PatternAdjust) = 0 THEN

39: ReducedROIZcount = 4

IF ErrorCode = 0 THEN

40: message = "A second attempt using a reduced threshold failed to find any focusable area for ROI " + ScanSlide.BaseName
41: ROIZ = SlideZ

ELSE

IF ErrorCode < 3 THEN

42: message = "A second attempt using a reduced threshold could not find a consensus focal plane for ROI " + ScanSlide.BaseName

43: ROIZ = ROIZ1

ELSE
END IF

END IF

ELSE
END IF

WEND
IF ErrorCode > 2 THEN

44: message "A second attempt using a reduced threshold successfully found multiple focusable areas for ROI " + ScanSlide.BaseName

ELSE
END IF
45: ROIZcount = 4
46: Log Variable(message, NEWLINE, NO HEADER)

\*\*\*End of Journal\*\*\*

**Supplemental Code 36. scan slide adjust.JNL**

1: SubZ = IF (FirstSubZ >= 1, ROIZ, SubZ)

2: TissuePresentDp = 0

3: TissuePresentFn = 0

IF DpFocChk > DpFocInt THEN

4: CurrentDpZ = IF (FirstSubZ >= 1, ROIZ, CurrentDpZ)

5: Device.Focus.CurPos = CurrentDpZ

6: FnFocChk = FnFocInt + 1

7: FocusChannel = DpFocCh

*Run Journal* C:\MM\app\mmproc\journals\122121 TMA scan\Load Autofocus Acquire Settings.JNL

8: Acquire()

9: DpFocExp = Image.Annotation

IF ReviseThreshold = 0 THEN

10: Scale Image([Last Result], AUTOSCALE, 50, 20)

11: TissuePresentDp = IF(Image.ScaleValueHigh - Image.ScaleValueLow > DpThreshold, 1, 0)

ELSE

12: Scale Image([8: Acquire], AUTOSCALE, 50, 10)

13: TissuePresentDp = IF(Image.ScaleValueHigh - Image.ScaleValueLow > ReducedDpThreshold, 1, 0)

END IF

14: Scale Image([Last Result], AUTOSCALE, 5, 2)

15: Close([Last Result])

IF TissuePresentDp = 1 THEN

16: Adjust Focus(11, 0.4, BACKLASH)

Number of Steps = (FirstSubZ \* (DpFocRange / DpStepSz)) + (DpFocRange / DpStepSz)

Step Size = DpStepSz

17: DpFocChk = 0

IF ABS(Device.Focus.CurPos - CurrentDpZ) <= (DpFocVar) THEN

18: CurrentDpZ = Device.Focus.CurPos

19: FirstSubZ = 0

ELSE

20: DpFocChk = DpFocInt + 1

IF ABS(Device.Focus.CurPos - CurrentDpZ) <= ((FirstSubZ + 1) \* DpFocVar) THEN

21: CurrentDpZ = Device.Focus.CurPos

22: FirstSubZ = 1

ELSE

23: FirstSubZ = FirstSubZ + 1

24: Device.Focus.CurPos = CurrentDpZ

END IF

END IF

ELSE

END IF

ELSE

END IF

IF FnFocChk > FnFocInt THEN

25: FocusChannel = FnFocCh

*Run Journal* C:\MM\app\mmproc\journals\122121 TMA scan\Load Autofocus Acquire Settings.JNL

26: Acquire()

27: FnFocExp = Image.Annotation

IF ReviseThreshold = 0 THEN

28: Scale Image([Last Result], AUTOSCALE, 50, 20)

29: TissuePresentFn = IF(Image.ScaleValueHigh - Image.ScaleValueLow > FnThreshold, 1, 0)

ELSE

30: Scale Image([Last Result], AUTOSCALE, 50, 10)

31: TissuePresentFn = IF(Image.ScaleValueHigh - Image.ScaleValueLow > ReducedFnThreshold, 1, 0)

END IF

32: Scale Image([Last Result], AUTOSCALE, 5, 2)

33: Close([Last Result])

IF TissuePresentFn = 1 THEN

34: FnFocChk = 0

35: Adjust Focus(11, 0.4, BACKLASH)

Number of Steps = (FirstSubZ \* (FnFocRange / FnFocStepSz)) + (FnFocRange / FnFocStepSz)

Step Size = FnFocStepSz

IF ABS(Device.Focus.CurPos - SubZ) <= FnFocVar THEN

36: SubZ = Device.Focus.CurPos

37: FirstSubZ = 0

ELSE

38: FnFocChk = FnFocInt + 1

IF ABS(Device.Focus.CurPos - SubZ) > ((FirstSubZ + 1) \* FnFocVar) THEN

39: DpFocChk = DpFocInt + 1

40: FirstSubZ = FirstSubZ + 1

ELSE

41: FirstSubZ = 1

42: SubZ = Device.Focus.CurPos

END IF

END IF

ELSE

END IF

ELSE

END IF

43: FnFocChk = FnFocChk + 1

44: DpFocChk = DpFocChk + 1

45: Device.Focus.CurPos = SubZ
\*\*\*End of Journal\*\*\*

**Supplemental Code 37. before each image set the Z.JNL**

IF ScanSlide.Status.WaveNum = 1 THEN

1: Device.Focus.CurPos = SubZ

ELSE

END IF

\*\*\*End of Journal\*\*\*

**Supplemental Code 38. Record variables.JNL**

1: Annotate Log File(EDGELIST, ".................................................................................")

2: Annotate Log File(EDGELIST, "Summary of TMA Scan Variables Settings")

3: Annotate Log File(EDGELIST, ".................................................................................")

4: message = "Time started:: " + StartTime

5: Log Variable(message, NEWLINE, NO HEADER)

6: message = "Time ended:: " + EndTime

7: Log Variable(message, NEWLINE, NO HEADER)

8: message = "Elasped time:: " + Stopwatches.Stopwatch1

9: Log Variable(message, NEWLINE, NO HEADER)

10: Annotate Log File(EDGELIST, ".................................................................................")

message = "New Stage list array.tif file created: " + NewArray

11: Log Variable(message, NEWLINE, NO HEADER)

12: Annotate Log File(EDGELIST, ".................................................................................")

13: Annotate Log File(EDGELIST, "Slide Layout")

message = "Number of slides: " + str(TotalSlideNumber)

14: Log Variable(message, NEWLINE, NO HEADER)

15: message = "First ROI position on Slide 1: " + Str(FirstROISlide1)

16: Log Variable(message, NEWLINE, NO HEADER)

17: message = "Focusing ROI for Slide 1: " + Str(FocusROISlide1)

18: Log Variable(message, NEWLINE, NO HEADER)

19: message = "Central Focal Plane for Slide 1: " + Str(Slide1Z) + " um"

20: Log Variable(message, NEWLINE, NO HEADER)

IF TotalSlideNumber > 1 THEN

21: message = "First ROI position on Slide 2: " + Str(FirstROISlide2)

22: Log Variable(message, NEWLINE, NO HEADER)

23: message = "Focusing ROI for Slide 2: " + Str(FocusROISlide2)

24: Log Variable(message, NEWLINE, NO HEADER)

25: message = "Central Focal Plane for Slide 2: " + Str(Slide2Z) + " um"

26: Log Variable(message, NEWLINE, NO HEADER)

ELSE

END IF

IF TotalSlideNumber > 2 THEN

27: message = "First ROI position on Slide 3: " + Str(FirstROISlide3)

28: Log Variable(message, NEWLINE, NO HEADER)

29: message = "Focusing ROI for Slide 3: " + Str(FocusROISlide3)

30: Log Variable(message, NEWLINE, NO HEADER)

31: message = "Central Focal Plane for Slide 3: " + Str(Slide3Z) + " um"

32: Log Variable(message, NEWLINE, NO HEADER)

ELSE

END IF

IF val(TotalSlideNumber) > 3 THEN

33: message = "First ROI position on Slide 4: " + Str(FirstROISlide4)

34: Log Variable(message, NEWLINE, NO HEADER)

35: message = "Focusing ROI for Slide 4: " + Str(FocusROISlide4)

36: Log Variable(message, NEWLINE, NO HEADER)

37: message = "Central Focal Plane for Slide 4: " + Str(Slide4Z) + " um"

38: Log Variable(message, NEWLINE, NO HEADER)

ELSE

END IF

39: Annotate Log File(EDGELIST, ".................................................................................")

40: Annotate Log File(EDGELIST, "Slide Focus Settings")

41: message = "Channel: " + str(TotalZFocCh)

42: Log Variable(message, NEWLINE, NO HEADER)

43: message = left(SlideZFocExp, (len(SlideZFocExp)-2))

44: Log Variable(message, NEWLINE, NO HEADER)

45: message = "Threshold values: " + Str(TotalZThreshold) + " and " + str(RepeatTotalZThreshold)

46: Log Variable(message, NEWLINE, NO HEADER)

47: message = "Maximum z range across all slides (um): " + str(TotalZRange)

48: Log Variable(message, NEWLINE, NO HEADER)

49: message = "Step size for determining central focal plan of each slide (um): " + str(TotalZFocStepSz)

50: Log Variable(message, NEWLINE, NO HEADER)

51: message = "Time started:: " + StartTime

message = "Maximum distance between points for central focal plane of each slide (um): " + str(TotalZFocVar)

52: Log Variable(message, NEWLINE, NO HEADER)

53: Annotate Log File(EDGELIST, ".................................................................................")

54: Annotate Log File(EDGELIST, "ROI Focus Settings")

55: message = "Channel: " + str(ROIZFocCh)

56: Log Variable(message, NEWLINE, NO HEADER)

57: message = left(ROIZFocExp, (len(ROIZFocExp)-2))

58: Log Variable(message, NEWLINE, NO HEADER)

59: message = "Threshold values: " + Str(ROIZThreshold) + " and " +Str(ReducedROIZThreshold)

60: Log Variable(message, NEWLINE, NO HEADER)

61: message = "Maximum z range within any single slide (um): " + Str(ROIZFocRange)

62: Log Variable(message, NEWLINE, NO HEADER)

63: message = "Step size for determining z origin of each ROI (um): " + Str(ROIZFocStepSz)

64: Log Variable(message, NEWLINE, NO HEADER)

65: message = "Maximum distance between points for z origin within each ROI (um): " + Str(ROIZFocVar)

66: Log Variable(message, NEWLINE, NO HEADER)

67: Annotate Log File(EDGELIST, ".................................................................................")

IF SSfocus = "Y" THEN

68: Annotate Log File(EDGELIST, "Interval Deep Focus Settings")

69: message = "Interval between deep focusing: " + Str(DpFocInt)

70: Log Variable(message, NEWLINE, NO HEADER)

71: message = "Channel: " + str(DpFocCh)
72: Log Variable(message, NEWLINE, NO HEADER)
73: message = left(DpFocExp, (len(DpFocExp)-2))
74: Log Variable(message, NEWLINE, NO HEADER)
75: message = "Threshold values: " + Str(DpThreshold) + " and " + Str(ReducedDpThreshold)
76: Log Variable(message, NEWLINE, NO HEADER)
77: message = "Deep focus z range (um): " + Str(DpFocRange)
78: Log Variable(message, NEWLINE, NO HEADER)
79: message = "Deep focus step size (um): " + Str(DpStepSz)
80: Log Variable(message, NEWLINE, NO HEADER)
81: message = "Maximum distance between old and new deep focus points (um): " + Str(DpFocVar)
82: Log Variable(message, NEWLINE, NO HEADER)
83: Annotate Log File(EDGELIST, ".................................................................................")
84: Annotate Log File(EDGELIST, "Interval Fine Focus Settings")
85: message = "Interval between fine focusing: " + Str(FnFocInt)
86: Log Variable(message, NEWLINE, NO HEADER)
87: message = "Channel: " + str(FnFocCh)
88: Log Variable(message, NEWLINE, NO HEADER)
89: message = left(FnFocExp, (len(FnFocExp)-2))
90: Log Variable(message, NEWLINE, NO HEADER)
91: message = "Threshold values: " + Str(FnThreshold) + " and " + Str(ReducedFnThreshold)
92: Log Variable(message, NEWLINE, NO HEADER)
93: message = "Fine focus z range (um): " + Str(FnFocRange)
94: Log Variable(message, NEWLINE, NO HEADER)

DpThresholdReducedDpThresholdDpFocRangeDpStepSzDpFocVarFnFocIntFnFocChFnThresholdReducedFnThresholdFnFocRangeFnFocStepSzFnFocVar95: message = "Fine focus step size (um): " + Str(FnFocStepSz)
96: Log Variable(message, NEWLINE, NO HEADER)
97: message = "Maximum distance between old and new fine focus points (um): " + Str(FnFocVar)
98: Log Variable(message, NEWLINE, NO HEADER)

ELSE

END IF

99: Annotate Log File(EDGELIST, ".................................................................................")

100: Annotate Log File(EDGELIST, ".................................................................................")

\*\*\*End of Journal\*\*\*

**Supplemental Code 39. Review Scan Summary.JNL**

1: Close Edge List Log()

2: Show Message and Wait("The log file located in the current Scan Slide save directory will be opened. Be sure the current save directory in Scan Slide is set to the directory to which images were saved during the TMA scan. If it is not corectly set press Cancel.", NO TIMEOUT)

3: tempdir = ScanSlide.Directory + "\settings\_and\_report.LOG"

4: Open Edge List Log(OPENFILE and APPENDMODE, "settings\_and\_report")

File Path = tempdir

5: View Current Edge List Log()

\*\*\*End of Journal\*\*\*

**Supplemental Code 40. Review Scans.JNL**

1: StartingScan = Enter Variable("Enter the first scan (ROI) number")
2: NumberofScans = Enter Variable("Enter the last scan (ROI) number")

3: Path = Enter Variable("Enter the full path for the scan file directory")

4: Path = if(right(Path,1)="\",Path, Path + "\")

5: Mag = Enter Variable("Choose the objective used for collecting the hi res image")

6: ROI = StartingScan
WHILE ROI < NumberofScans DO

7: ScanName = Path + "Scan " + mag + " ROI " + str(ROI) + ".scan"
8: Does File Exist?("%ScanName%")
IF DoesFileExist.ErrorCode = 0 THEN

9: "Scan 20x ROI 2" = Open Scan()

Scan File Name = ScanName
10: *Loop for all Images*("set image zoom and scaling")
11: Show Message and Wait("Press continue when finished reviewing this low resolution view of %ScanName%.", NO TIMEOUT)
12: Close All(NOQUERY)

ELSE

END IF
13: ROI = ROI + 1
WEND

\*\*\*End of Journal\*\*\*

**Supplemental Code 41. LoadSaveRescanSettings.JNL**

1: LoadSave = Enter Variable("Are TMA rescan settings being loaded or saved?")

IF LoadSave = "load" THEN

2: "TMA rescan current settings" = Scan Slide Load Settings(ACQUISITION SETTINGS and CALIBRATIONS)

ELSE

END IF

IF LoadSave = "save" THEN

3: "TMA rescan current settings" = Scan Slide Save Settings()

ELSE

END IF

\*\*\*End of Journal\*\*\*

**Supplemental Code 42. set image zoom and scaling.JNL**

1: Set Image Zoom([Current At Start], 40)
2: Scale Image([Current At Start], AUTOSCALE, 4, 1)

\*\*\*End of Journal\*\*\*

**Supplemental Code 43. Stitch Stack and Save Images.JNL**

1: StartingScan = Enter Variable("Enter the first scan (ROI) number")
2: NumberofScans = Enter Variable("Enter the last scan (ROI) number")

3: Path = Enter Variable("Enter the full path for the scan file directory")

4: Path = if(right(Path,1)="\",Path, Path + "\")
5: NewDirectory = Path + "stitched\_stacks\"
6: Create Directory("%newdirectory%")

.
7: Mag = Enter Variable("Choose the objective used for collecting the hi res image")

8: ROI = StartingScan
WHILE ROI < NumberofScans DO

9: ScanName = Path + "Scan " + mag + " ROI " + str(ROI) + ".scan"
10: Does File Exist?("%ScanName%")
IF DoesFileExist.ErrorCode = 0 THEN

11: "Scan 20x ROI 2" = Open Scan()

Scan File Name = ScanName
12: Scan Slide Show High Resolution Image: Zoom Percent = 100

IF ImageExists("637-684 Selection") THEN
13: Add Plane("637-684 Selection", "Stack of stitched images", CLOSESOURCE)
ELSE
END IF
IF ImageExists("575-615 Selection") THEN
14: Add Plane("575-615 Selection", "Stack of stitched images", CLOSESOURCE)
ELSE
END IF

IF ImageExists("488-527 Selection") THEN
15: Add Plane("488-527 Selection", "Stack of stitched images", CLOSESOURCE)
ELSE
END IF
IF ImageExists("360-440 Selection") THEN
16: Add Plane("360-440 Selection", "Stack of stitched images", CLOSESOURCE)
ELSE
END IF

17: Select Image("Stack of stitched images")
18: Image.FilePath = NewDirectory + "Scan " + mag + " ROI " +str(ROI)+ ".TIF"
19: Save("Stack of stitched images")
20: Close("Stack of stitched images")

ELSE
END IF

21: ROI = ROI + 1
WEND

\*\*\*End of Journal\*\*\*