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MetaXpress[®] Software: *Mitotic Index Module*



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Mitotic Index Module Overview

📽 Configure Settings for Mite	otic Index 📃 🗆 🔀
⊖All nuclei	
W1 Source image:	DAPI Adaptive
Display result image:	[None] Background Correction TM system
Algorithm:	Fast 🗸
Approximate min width:	5 🗢 μm = 16 pixels
Approximate max width:	10 🗢 μm = 31 pixels
Intensity above local background:	300 📚 graylevels Preview
 Mitotic staining 	
W2 Source image:	FITC
Intensity above local background:	300 📚 graylevels Preview
Configure Summary Log	Configure Data Log (Cells)
Save Settings Load Settings	Set to Defaults Test Run Close

- The Mitotic Index module can be used to analyze cellular images to differentiate between Mitotic and Interphase cells in a normal cell cycle.
- This module requires a nuclear wavelength and mitotic-specific stain wavelength.
- A typical mitotic-specific stain used for this module is Histone 3
 S10 phosphorylation. DNA stain labels all cells and the mitotic cells are labeled only with the mitoticspecific stain.



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Mitotic Index – Example images



Interphase nuclei are shown with a red overlay and mitotic nuclei with a green overlay.

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Nuclear Stain

Mitotic-Specific Stain

Module Settings – Nuclei classification

📽 Configure Settings for Mite	otic Index 📃 🗖 🔀
All nuclei W1 Source image: Display result image:	DAPI Adaptive Background [None] Correction TM system
Algorithm:	Fast 💌
Approximate min width:	5 🗢 μm = 16 pixels
Approximate max width:	10 🗢 μm = 31 pixels
Intensity above local background:	300 📚 graylevels Preview
Mitotic staining W2 Source image: Intensity above local background:	FITC 300 📚 graylevels Preview
Configure Summary Log	Configure Data Log (Cells)
Save Settings Load Settings	Set to Defaults Test Run Close

Nuclei classification





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2. Module Settings – DNA content

📽 Configure Settings for Mito	otic Index
All nuclei W1 Source image: Display result image:	DAPI Adaptive Background [None] Correction TM system
Algorithm:	Fast 💌
Approximate min width:	5 🗢 μm = 16 pixels
Approximate max width:	10 🤤 μm = 31 pixels
Intensity above local background:	300 🗢 graylevels Preview
Mitotic staining W2 Source image:	FITC
Intensity above local background:	300 🗢 graylevels Preview
Configure Summary Log	Configure Data Log (Cells)
Save Settings Load Settings	Set to Defaults Test Run Close

 Select the wavelength for the DNA content (nuclear stain)



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1. Module Settings – result image

📽 Configure Settings for Mite	otic Index 📃 🗆 🔀
All nuclei W1 Source image:	DAPI Adaptive Background
Display result image:	[None] Correction" system
Approximate min width:	5 μm = 16 pixels
Approximate max width: Intensity above local background:	10
Mitotic staining W2 Source image:	FITC
Intensity above local background:	300 🗢 graylevels Preview
Configure Summary Log	Configure Data Log (Cells)
Save Settings Load Settings	Set to Defaults Test Run Close

 Leave "Display result image" deselected (this is generally only used when journaling)



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Module Settings

🖉 Configure Settings for Mitotic Index		
All nuclei W1 Source image: DAPI Background Display result image: [None] System		
Algorithm: Fast ✓ Approximate min width: Standard Fast Approximate max width: 10 ↓ µm = 31 pixels Intensity above local background: 300 ≩ graylevels Preview		
Mitotic staining W2 Source image: FITC Intensity above local background: 300 🜍 graylevels Preview		
Configure Summary Log Configure Data Log (Cells) Save Settings Load Settings Set to Defaults Test Run Close		

Algorithm

- This option is only available in MetaXpress software version 4.0 and higher and determines how quickly the analysis is performed.
- Fast algorithm can perform analysis up to twice as fast as Standard.
- Both algorithms produce similar but not identical results.



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3. Module Settings – width settings

🜌 Configure Settings for Mitot	tic Index 📃 🗖 🔀		
All nuclei W1 Source image: D Display result image:	DAPI Adaptive Background [None] Correction TM system		
Algorithm: F Approximate min width: 5 Approximate max width: 1	Fast 5		
Intensity above local background: 300 graylevels Preview Mitotic staining W2 Source image: FITC			
Intensity above local background: 300 graylevels Preview Configure Summary Log Configure Data Log (Cells) Save Settings Set to Defaults Test Run Close			



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- Set the Approximate min width and Approximate max width for the range of nuclei that you want to detect
- The width is the short axis of a nucleus (in um).
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split



3. Module Settings – width settings

Effects of varying width settings

Min width too small: splits nuclei

Min width too large: omits smaller nuclei

Max width too small: may shrink nuclear boundaries

Max width too large: may slightly enlarge nuclear boundaries

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Module Settings – Intensity above background

Configure Settings for Mito	otic Index 📃 🗆 🔀	
All nuclei		
W1 Source image:	DAPI Adaptive	L
Display result image:	[None] Background Correction TM system	L
Algorithm:	Fast 🗸	L
Approximate min width:	5 🗢 μm = 16 pixels	L
Approximate max width:	10 🗢 μm = 31 pixels	
Intensity above local background:	300 🗢 graylevels Preview	
And the second second		
W2 Source image:	FITC	
Intensity above local background:	300 📚 graylevels Preview	L
Configure Summary Log	Configure Data Log (Cells)	L
1024 -		
0+) 7.05 13.09 19.14 Distance (um)	

- The intensity above local background is used for finding the nuclei
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim cell and its local background.
 For FAST algorithm, set this value to about half (or less) of the difference in intensity between a dim cell and local background.
 - Draw a line across a cell into the background and use Measure > Linescan to determine intensity values; or simply mouse over the cell and the background and view the intensity values



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4. Module Settings – Nuclei segmentation

Configure Settings for Mite	otic Index	
All nuclei	DAR	1 dantésa
W1 Source image:	DAPI	Background
Display result image:	[None]	Correction''' system
Algorithm:	Fast 🔽	
Approximate min width:	5 🗢 μm = 16 pixels	
Approximate max width:	10 🗢 μm = 31 pixels	
Intensity above local background:	300 📚 graylevels	Preview
Mitotic staining		
W2 Source image:	FITC	
Intensity above local background:	300 🤤 graylevels	Preview
Configure Summary Log	Configure Data Log (Cel	ls)
Save Settings Load Settings	Set to Defaults Tes	t Run Close

- Click on **Preview** to test the segmentation settings for the nuclei
- The Preview image will show a red overlay over the segmentated nuclei. The overlay can be toggled on and off.





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5. Module Settings – Mitotic Cell classification

📽 Configure Settings for Mite	otic Index	
All nuclei W1 Source image: Display result image:	DAPI [None]	Adaptive Background Correction [™] system
Algorithm:	Fast 💌	
Approximate min width:	5 🛟 μm = 16 pixels	
Approximate max width:	10 🛟 μm = 31 pixels	
Intensity above local background:	300 🤤 graylevels	Preview
Mitotic staining W2 Source image: Intensity above local background:	FITC graylevels	Preview
Configure Summary Log	Configure Data Log (Cells)	
Save Settings Load Settings	Set to Defaults Test Ru	n Close

Mitotic classification

 The average intensity (brightness) of the mitotic-specific marker (e.g. phospho-histone H3) is used to identify mitotic cells.





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6. Module Settings – mitotic classification

📽 Configure Settings for Mite	otic Index 📃 🗆 🔀		
⊖ All nuclei			
W1 Source image:	DAPI Adaptive Background		
Display result image:	[None] Correction [™] system		
Algorithm:	Fast 🗸		
Approximate min width:	5 🔿 μm = 16 pixels		
Approximate max width:	10 🗢 μm = 31 pixels		
Intensity above local background:	300 🗢 graylevels Preview		
Mitotic staiping			
W2 Source image:	FITC		
Intensity above local background:	300 📚 graylevels Preview		
Configure Summary Log	Configure Data Log (Cells)		
Save Settings Load Settings	Set to Defaults Test Run Close		

- Mitotic-specific stain
- Select the wavelength for the mitotic marker



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7. Module Settings – mitotic classification

📽 Configure Settings for Mitotic	: Index		
_ All nuclei			
W1 Source image: DA	PI	Adaptive Background	
🗌 Display result image: 🔢	one]	Correction [™] system	
Algorithm: Fa	st 💌		
Approximate min width: 5	🜲 μm = 16 pixels		
Approximate max width: 10	🗢 μm = 31 pixels		
Intensity above local background: 300) ᅌ graylevels	Preview	
Mitotic staining			
W2 Source image: FIT	10		
Intensity above local background: 30	0 ᅌ graylevels	Preview	
	Markan Secon		- [
Configure Summary Log	Source Image: FITC		
	Lines	can on FITC	
	717 -	\land	
	vel (Avg)		
	Gray Le		
	364 -		
Tagakhan khrough life agissoo	187	17.50 25.75	34.
IOUEUTER UTROUCH INE SCIENCES		exercise (anny	

- Enter a cutoff intensity value for the mitotic specific stain.
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim mitotic cell and its local background. For FAST algorithm, set this value to about half (or less) of the difference in intensity between a dim cell and local background.
- Draw a line across a cell into the background and use Measure > Linescan to determine intensity values; or simply mouse over the cell and the background and view the intensity values.



8. Module Settings – mitotic classification

Configure Settings for Mite	otic Index	
⊖ All nuclei		
W1 Source image:	DAPI	Adaptive Background
Display result image:	[None]	Correction [™] system
Algorithm:	Fast 💌	
Approximate min width:	5 🗢 🗢 μm = 16 pixels	
Approximate max width:	10 🗢 μm = 31 pixels	
Intensity above local background:	300 🤤 graylevels	Preview
Mitotic staining		
W2 Source image:	FITC	
Intensity above local background:	300 🔹 graylevels	Preview
Configure Summary Log	Configure Data Log (Cell:	s)
Save Settings Load Settings	Set to Defaults Test	Run Close

- Press Preview to see cells with that level of staining highlighted in the image.
- The Preview image will show a green overlay on the mitotic staining image to show the segmentation derived from the image. The overlay can be toggled on and off.





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9. Module Settings – final settings

Configure Settings for Mite	otic Index	
All nuclei		
W1 Source image:	DAPI	Adaptive Restructure
Display result image:	[None]	Background Correction™ system
Algorithm:	Fast 🐱	
Approximate min width:	5 文 μm = 16 pixels	
Approximate max width:	10 文 μm = 31 pixels	
Intensity above local background:	300 ᅌ graylevels	Preview
Mitotic staining		
W2 Source image:	FITC	
Intensity above local background:	300 ᅌ graylevels	Preview
Configure Summary Log	Configure Data Log (Ce	ells)
Save Settings Load Settings	Set to Defaults Te	st Run Close

- Configure Summary Log select site-by-site measurements
- Configure Data Log select cell-bycell measurements
- Save Settings save analysis parameters to database
- Load Settings load saved analysis parameters
- Set to Defaults restore default analysis parameters
- Test Run test all settings together and display cell-by-cell results for this site

Molecular Devices

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- 🧹 Image Name
- 🗸 🛛 Image Plane
- Image Date and Time
- Elapsed Time
- 🧹 Stage Label
- 🗸 Wavelength
- Z Position
- 🧹 Total Nuclei
- Mitotic Nuclei
- Interphase Nuclei
- 🧹 % Mitotic Nuclei
- 🧹 🕺 Interphase Nuclei
- All Nuclei Total Area
- 🖌 All Nuclei Mean Area
- All Nuclei W1 Integrated Intensity
- All Nuclei W1 Average Intensity
- All Nuclei W2 Integrated Intensity
- All Nuclei W2 Average Intensity
- Mitotic Total Area
- 🖌 Mitotic Mean Area
- Mitotic W1 Integrated Intensity
- Mitotic W1 Average Intensity
- Mitotic W2 Integrated Intensity
- Mitotic W2 Average Intensity
- Interphase Total Area
- Interphase Mean Area
- Interphase W1 Integrated Intensity
- Interphase W1 Average Intensity
- Interphase W2 Integrated Intensity
- Interphase W2 Average Intensity

Summary or site-by-site measurements are for the entire image and include:

• Total Nuclei:

Total number cells determined from wavelength 1.

• Mitotic Nuclei:

Total number nuclei determined from wavelength 1 that were positive in wavelength 2.

Interphase Nuclei:

Total number nuclei determined from wavelength 1 that were negative in wavelength 2.

- % Mitotic Nuclei:
- 100 * Mitotic Nuclei/Total Nuclei.
- % Interphase Nuclei:
- 100 * Interphase Nuclei/Total Nuclei.





Summary or site-by-site measurements are for the entire image and include:

- Image Name
- Image Plane
- Image Date and Time
- Elapsed Time
- Stage Label
- Wavelength
- Z Position
- Total Nuclei
- Mitotic Nuclei
- Interphase Nuclei
- % Mitotic Nuclei
- % Interphase Nuclei
- All Nuclei Total Area
- All Nuclei Mean Area
- All Nuclei W1 Integrated Intensity
- All Nuclei W1 Average Intensity
- All Nuclei W2 Integrated Intensity
- All Nuclei W2 Average Intensity
- Mitotic Total Area
- Mitotic Mean Area
- Mitotic W1 Integrated Intensity
- Mitotic W1 Average Intensity
- Mitotic W2 Integrated Intensity
- Mitotic W2 Average Intensity
- Interphase Total Area
- Interphase Mean Area
- Interphase W1 Integrated Intensity
- Interphase W1 Average Intensity
- Interphase W2 Integrated Intensity
- Interphase W2 Average Intensity

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- All Nuclei Total Area:
- Total μ m²s in wavelength 1 in all nuclei.
- All Nuclei Mean Area:

All Nuclei Total Area/Total Nuclei.

All Nuclei W1 Integrated Intensity:

Summed grayscale values in wavelength 1 in all nuclei.

All Nuclei W1 Average Intensity:

All Nuclei Integrated Intensity/All Nuclei Total (Pixel) Area.

All Nuclei W2 Integrated Intensity:

Summed grayscale values in wavelength 2 in all nuclei.

All Nuclei W2 Average Intensity:

All Nuclei W2 Integrated Intensity/All Nuclei Total (Pixel) Area.



🧹 Image Name

- 🗸 🛛 Image Plane
- Image Date and Time
- Elapsed Time
- 🧹 Stage Label
- 🗸 Wavelength
- Z Position
- 🖌 Total Nuclei
- Mitotic Nuclei
- Interphase Nuclei
- 🧹 🕺 Mitotic Nuclei
- 🗸 🕺 Interphase Nuclei
- All Nuclei Total Area
- 🖌 All Nuclei Mean Area
- All Nuclei W1 Integrated Intensity
- All Nuclei W1 Average Intensity
- All Nuclei W2 Integrated Intensity
- All Nuclei W2 Average Intensity
- Mitotic Total Area
- Mitotic Mean Area
- Mitotic W1 Integrated Intensity
- Mitotic W1 Average Intensity
- Mitotic W2 Integrated Intensity
- Mitotic W2 Average Intensity
- Interphase Total Area
- Interphase Mean Area
- Interphase W1 Integrated Intensity
- Interphase W1 Average Intensity
- Interphase W2 Integrated Intensity
- Interphase W2 Average Intensity

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• Mitotic Total Area:

Total μ m²s in wavelength 1 in mitotic nuclei.

• Mitotic Mean Area:

Mitotic Total Area/Mitotic Nuclei.

• Mitotic W1 Integrated Intensity:

Summed grayscale values in wavelength 1 in mitotic nuclei.

• Mitotic W1 Average Intensity:

Mitotic Nuclei W1 Integrated Intensity/Mitotic Nuclei Total (Pixel) Area.

Mitotic W2 Integrated Intensity:

Summed grayscale values in wavelength 2 in mitotic nuclei.

• Mitotic W2 Average Intensity:

Mitotic W2 Integrated Intensity/Mitotic Total (Pixel) Area.



🧹 Image Name

- Image Plane
- Image Date and Time
- Elapsed Time
- 🧹 Stage Label
- 🗸 Wavelength
- Z Position
- Total Nuclei
- Mitotic Nuclei
- Interphase Nuclei
- 🧹 🕺 Mitotic Nuclei
- 🗸 % Interphase Nuclei
- All Nuclei Total Area
- 🖌 All Nuclei Mean Area
- All Nuclei W1 Integrated Intensity
- All Nuclei W1 Average Intensity
- All Nuclei W2 Integrated Intensity
- All Nuclei W2 Average Intensity
- Mitotic Total Area
- Mitotic Mean Area
- Mitotic W1 Integrated Intensity
- Mitotic W1 Average Intensity
- Mitotic W2 Integrated Intensity
- Mitotic W2 Average Intensity
- Interphase Total Area
- 🖌 Interphase Mean Area
- Interphase W1 Integrated Intensity
- Interphase W1 Average Intensity
- Interphase W2 Integrated Intensity
- Interphase W2 Average Intensity

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• Interphase Total Area:

Total μ m²s in wavelength 1 in interphase nuclei.

• Interphase Mean Area:

Interphase Total Area/Interphase Nuclei.

Interphase W1 Integrated Intensity:

Summed grayscale values in wavelength 1 in interphase nuclei.

• Interphase W1 Average Intensity:

Interphase W1 Integrated Intensity/Interphase Total (Pixel) Area.

Interphase W2 Integrated Intensity:

Summed grayscale values in wavelength 2 in interphase nuclei.

Interphase W2 Average Intensity:

Interphase W2 Integrated Intensity/Interphase Total (Pixel) Area.



Cell Data (cell-by-cell measurements)

- Image Name
- Image Plane
- Image Date and Time
- Elapsed Time
- Stage Label
- Wavelength
- Z Position
- Cell: Assigned Label #
- Cell: Mitotic Classification
- 🧹 Cell: Total Area
- Cell: W1 Integrated Intensity
- Cell: W1 Average Intensity
- Cell: W2 Integrated Intensity
- Cell: W2 Average Intensity

- Data Log (Cell-by-Cell Measurement) For each nucleus, the following measurements are included in the Data Log:
- Cell: Assigned Label #:
- Cell label number (1 through total cell number)
- **Cell: Classification:** Interphase, Mitotic
- Cell: Total Area:
- Total $\mu m^2 s$ in the nucleus.



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Cell Data (cell-by-cell measurements)

- Image Name
- Image Plane
- Image Date and Time
- Elapsed Time
- Stage Label
- Wavelength
- Z Position
- Cell: Assigned Label #
- Cell: Mitotic Classification
- 🧹 Cell: Total Area
- Cell: W1 Integrated Intensity
- Cell: W1 Average Intensity
- Cell: W2 Integrated Intensity
- Cell: W2 Average Intensity

• Data Log (Cell-by-Cell Measurement): For each nucleus, the following measurements are included in the Data Log:

• Cell: W1 Integrated Intensity:

Summed grayscale values in wavelength 1 for this nucleus.

Cell: W1 Average Intensity:

W1 Integrated Intensity/Total (Pixel) Area

• Cell: W2 Integrated Intensity:

Summed grayscale values in wavelength 2 for this nucleus.

Cell: W2 Average Intensity:

W2 Integrated Intensity/Total (Pixel) Area



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