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MetaXpress[®] Software: Cell Scoring Module



Cell Scoring Module Overview

- The Cell Scoring module can be used to analyze cells imaged in 2 wavelengths
- W1 should be a stain for all nuclei (e.g. DAPI, Hoechst, or DRAQ5)
- W2 is the stain of interest for scoring the cells
- The module will score cells as positive (green) or negative (red) for W2





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| W1 Source image: | DAPI | | | Adaptive Rockmound |
|-----------------------------------|----------|----|-----------------|--------------------------|
| 🔲 Display result image: | [None] | | | Correction TM |
| Approximate min width: | 8 | ÷ | µm = 8 pixels | system |
| Approximate max width: | 25 | ÷ | µm = 25 pixels | |
| Intensity above local background: | 500 | ÷ | graylevels | Preview |
| Stained area: | Cytoplas | sm | ▼ | |
| Stained area: | Cytoplas | sm | _ | |
| Approximate min width: | 100 | - | µm = 10 pixels | |
| Approximate max width: | 100 | - | µm = 101 poceis | 1 |
| Intensity above local background: | 1000 | Ī | graylevels | Preview |
| gorithm: Fast 💌 | I | | | |
| | 1 | | | |

• Display result image

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

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| W1 Source imag Display result imag | e: DAPI | _ | Ada Bao Cor sys | nptive kground rection TM tem |
|--|--|--|--------------------------|---|
| Approximate min widt | h: 8 | µm = 8 pixels | | |
| Approximate max widt | h: 25 📑 | µm = 25 pixels | | |
| Intensity above local backgroun | d: 500 📑 | graylevels | Preview | / |
| Stained are Approximate min widt Approximate max widt Intensity above local backgroun | a: Cytoplasm h: 10 h: 100 d: 1000 | עmr = 10 pixels μmr = 101 pixe graylevels | ls Preview | <u>. </u> |
| Ngorithm: Fast | • | | | |
| Configure Summary Log | Config | gure Data Log (C | ells) | |
| | | the second s | | |

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.



| lgorithm: | Fast | - | | | |
|------------|-----------------------|----------------|-----------|----------|-----------------------------------|
| Intensity | above local backg | round: 1000 | ÷ graylev | els | Preview |
| | Approximate max | width: 100 | ÷ µm = 10 | 1 pixels | |
| | Approximate min | width: 10 | ÷ µm = 10 |) pixels | |
| | Stained | d area: Cytop! | asm | - | |
| Positive m | narker W2 Source i | image: FITC | | | |
| Intensity | above local backg | round: 500 | 🕂 graylev | els | Preview |
| | Approximate max | width: 25 | ÷ µm = 2 | 5 pixels | |
| | Approximate min | width: 8 | ± μm = 8 | pixels | |
| | Display result i | image: [None | 1 | | Correction [™] system |
| | W1 Source i | image: DAPI | | | Adaptive Background |

- Wavelength 1 (W1)
- Select the wavelength for the nuclei
- Do not select the "HTS" image (thumbnail montage)



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| All nuclei W1 Source image: Display result image: | DAPI [None] | | | Adaptive Background Correction [™] system |
|---|----------------|--------|---------------------------------|---|
| Approximate min width: Approximate max width: | 8 25 | + + | µm = 8 pixels µm = 25 pixels | |
| Intensity above local background: | 500 | ÷ | graylevels | Preview |
| Positive marker | | | | |
| W2 Source image: | FITC | | | |
| Stained area: | Cytoplas | sm | - | |
| Approximate min width: | 10 | + | µm = 10 pixels | |
| Approximate max width: | 100 | ÷ | µm = 101 pixels | |
| Intensity above local background: | 1000 | • | graylevels | Preview |
| Ngorithm: Fast <u> Configure Summary Log</u> | | | | |
| Save Settings | s | | | |
| | | | | |
| | | | / | |
| | | | X: 410 | Y: 334 |

Wavelength 1 (W1)

- 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



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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





| Г | W1 Source Display result | image: DAPI | J 1 | | Adaptive Background Correction [™] system | |
|-----------------------|-----------------------------|-------------|-----------|-----------|---|--|
| Ap | proximate min | width: 8 | ÷ µm = 8 | pixels | -, | |
| Ap | proximate max | width: 25 | ÷ µm = 2 | 5 pixels | | |
| Intensity abov | ve local backg | round: 500 | 🕂 graylev | rels Pr | eview | |
| Positive marks | w. | | | | | |
| USILIVE IIIdIKE | W2 Source | mage FITC | 1 | | | |
| | Stainer | larea: Otor | J | • | | |
| Ar | noroximate min | width 10 | -1 um = 1 | 0 pixels | | |
| An | provinate may | width: 100 | - um - 1 | 01 nivels | | |
| Intensity abov | ve local backo | round: 1000 | | rele Pr | eview | |
| and the second second | | | - grafier | | | |
| | Gray Level | 048 - | | | | |
| | | ~~~~ | | | | |
| | 1 | 024 - | | | | |
| | | | | | | |
| | | | | 5 D. | 84. 38 S | |
| | | 1.00 | 7.05 | 13.09 | 19.14 | |

Wavelength 1 (W1)

- 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 (Standard algorithm)
- If using the Fast algorithm, set intensity value to about half of this

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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| W1 Source image: | DAPI [None] | | | Adaptive Backgroi Correctio | Ind TM |
|-----------------------------------|----------------|---------------------------------------|----------------|-----------------------------------|-----------|
| Approximate min width: | 8 | | 8 nivels | system | |
| Approximate max width: | 25 | · · · · · · · · · · · · · · · · · · · | 25 pixels | | |
| Intensity above local background: | 500 | ⇒ grayle | vels | Preview | |
| Stained area: | Cytoplasm | n Al um | ▼ 10 single | | |
| Approximate min width: | 10 | ÷ µm = | 10 pixels | | |
| Approximate max width: | 100 | ± μm = | 101 pixels | | |
| Intensity above local background: | 1000 | 🛨 grayle | vels | Preview | |
| East = | | | | | 1 |
| Igonthm: Jrasi | | | | | |

Wavelength 1 (W1)

 Click on **Preview** to test settings for the current wavelength only

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| W1 Source image: | DAPI | | Adaptive |
|--|--|--|---------------------------------------|
| Display result image: | [None] | | Background Correction [™] |
| A | | 0 | system |
| Approximate min width: | P | µm = 8 pixels | |
| Approximate max width: | 25 ÷ | µm = 25 pixels | |
| Intensity above local background: | 500 🔶 | graylevels | Preview |
| - Positive marker W2 Source image: Stained area: | FITC | | |
| Positive marker W2 Source image: Stained area: | FITC Cytoplasm | _ | |
| Positive marker W2 Source image: Stained area: Approximate min width: | FITC Cytoplasm | _ μm = 10 pixels | |
| Positive marker W2 Source image: Stained area: Approximate min width: Approximate max width: | FITC Cytoplasm 10 ÷ 100 ÷ | עm = 10 pixels μm = 101 pixels | |
| Positive marker W2 Source image: Stained area: Approximate min width: Approximate max width: Intensity above local background: | FITC Cytoplasm 10 ÷ 100 ÷ 1000 ÷ | ↓m = 10 pixels µm = 101 pixels graylevels | Preview |
| Positive marker W2 Source image: Stained area: Approximate min width: Approximate max width: Intensity above local background: Algorithm: Fast | FITC Cytoplasm 10 ÷ 100 ÷ 1000 ÷ | _ μm = 10 pixels μm = 101 pixels graylevels | Preview |

Wavelength 2 (W2)

- Select wavelength for the marker stain
- Do not select the "HTS" image (thumbnail montage)



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| W | 1 Source image: _ | DAPI | | Adaptive Background |
|---------------------|----------------------|----------|-----------------|------------------------|
| I Disp | lay result image: [[| None] | | system |
| Approx | imate min width: 8 | ÷ | µm = 8 pixels | |
| Approxi | mate max width: 2 | 5 🕂 | µm = 25 pixels | |
| Intensity above loo | cal background: 5 | 00 ÷ | graylevels | Preview |
| Anomy | imate min width: 1 | ytopiasm | um = 10 pixels | J |
| Approx | imate min width: 1 | 0 🗄 | µm = 10 pixels | |
| Approxi | mate max width: 1 | 00 ÷ | µm = 101 pixels | |
| Intensity above loo | cal background: 1 | 000 ÷ | graylevels | Preview |
| | - | | | |
| Igorithm: Fast | | | | |

Wavelength 2 (W2)

• Define Stained area:

- Nucleus (stain matches W1 area)
- Cytoplasm (stain is surrounding but not including W1 area)
- Nucleus and Cytoplasm (stain encompasses W1 and surrounding area)



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Stained Area



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| , and doior | W1 Source image: | DAPI | | Adaptive Background |
|-------------|-------------------------|-----------|-------------------|------------------------------------|
| | Display result image: | [None] | | Correction TM system |
| | Approximate min width: | 8 | μm = 8 pixels | 0,000 |
| | Approximate max width: | 25 | ÷ μm = 25 pixels | |
| Intensity | above local background: | 500 - | graylevels | Preview |
| | Stained area: | Cytoplasm | ⊥ um = 10 nivels | |
| | Approximate min width: | 10 | ∲µm = 10 pixels | |
| | Approximate max width: | 100 | μm = 101 pixel | s |
| Intensity | above local background: | 1000 | graylevels | Preview |
| Ngorithm: | Fast 💌 |] | | |
| Cart | Summer Les | 1 Carl | iouro Doto Los (C | |

Wavelength 2 (W2)

- Set the Approximate min width and Approximate max width for the stain of interest
- For a nuclear stain, use the same settings as W1
- For a cytoplasmic or nuclear + cytoplasmic stain, measure the whole cell the same way you measured the nucleus for W1



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| W1 Source image: | DAPI | | Adaptive |
|-----------------------------------|-----------|-----------------------|--|
| E Diselau see dhimage. | Blanal | | Background Correction TM |
| Display result image: | [None] | | system |
| Approximate min width: | 8 🗄 | µm = 8 pixels | |
| Approximate max width: | 25 ÷ | µm = 25 pixels | |
| Intensity above local background: | 500 ÷ | graylevels | Preview |
| - Positive marker | | | |
| W2 Source image: | FITC | | |
| Stained area: | Cytoplasm | • | |
| Approximate min width: | 10 🔶 | µm = 10 pixels | |
| Approximate max width: | 100 ÷ | µm = 101 pixels | |
| Intensity above local background: | 1000 ÷ | graylevels | Preview |
| Algorithm: Fast 💌 | [| | |
| Configure Summary Log | Configu | ire Data Log (Cells). | |
| Cours Comings 1 Lond Comings | Catta Da | (| - Cross |

Wavelength 2 (W2)

- Intensity above local background for the stain of interest is used to distinguish positive from negative cells
- Measure the stain intensity vs background just as for W1
- Note that the optimal intensity setting for the Fast algorithm is about half of the optimal setting for the Standard algorithm
- Check both positive and negative control images to help set a suitable cutoff



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| DAPI [None] | | Adaptive Background Correction™ |
|----------------|--|--|
| 8 ÷ | µm = 8 pixels | system |
| 25 ÷ | µm = 25 pixels | |
| 500 ÷ | graylevels | Preview |
| 10 ÷ | µm = 10 pixels | |
| 10 ÷ | µm = 10 pixels | |
| 100 🚖 | µm = 101 pixels | |
| 1000 🛨 | graylevels | Preview |
| | | |
| | | |
| | DAPI [None] 8 € 25 € 500 € FITC Cytoplasm 10 100 € 1000 € | DAPI [None] 8 25 500 graylevels FITC Cytoplasm 10 100 |

Wavelength 2 (W2)

- Click on **Preview** to test settings for the current wavelength only
- Note that this may not accurately segment the cells as this Preview does not use the nuclear staining
- This is most useful for testing the Intensity settings for this wavelength



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Module Settings – General Settings

| | | Background |
|-----------|--|------------|
| [None] | | system |
| 8 ÷ | µm = 8 pixels | |
| 25 ÷ | µm = 25 pixels | |
| 500 ÷ | graylevels | Preview |
| Cytoplasm | _ | |
| Cytoplasm | _ | |
| | µm = 10 poxeis | |
| 100 - | µm = 101 pixels | |
| 1000 🛨 | graylevels | Preview |
| | | |
| Configu | re Data Log (Cells) | |
| | 3 1 25 1 500 1 FITC 1 Cytoplasm 1 100 1 1000 1 1000 1 1000 1 | 3 |

- 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



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- **Total cells:** Total number of nuclei (cell count)
- **Positive Cells:** The total number of cells positive for staining with wavelength 2 as defined in the settings
- Negative Cells: The total number of cells negative for staining with wavelength 2 as defined in the settings
- % Positive Cells: The number of positive W2 cells divided by the total number of cells, times 100
- % Negative Cells: The number of negative W2 cells divided by the total number of cells, times 100



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- All Nuclei Total Area: The total area of nucleus for all cells found in the image (in um²)
- All Nuclei Mean Area: The average area of nucleus for all cells found in the image (in um²)
- All Nuclei W1 Integrated Intensity: The total pixel intensity of the nuclear stain over the nuclear area
- All Nuclei W1 Average Intensity: The average pixel intensity of the nuclear stain over all the nuclear areas in the image
- All Nuclei W2 Integrated Intensity: The total pixel intensity of the marker stain over the nuclear area
- All Nuclei W2 Average Intensity: The average pixel intensity of the marker stain over all the nuclear areas in the image



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- **Positive Cells Total Area:** The total cell area for all positive cells found in the image (in um²)
- Positive Cells Mean Area: The average cell area for all positive cells found in the image (in um²)
- **Positive Cells W2 Integrated Intensity:** The total pixel intensity of the marker stain over the cell area
- Positive Cells W2 Average Intensity: The average pixel intensity of the marker stain over all the cell areas in the image



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- Positive Nuclei Total Area: The total area of nucleus for positive cells found in the image (in um²)
- Positive Nuclei Mean Area: The average area of nucleus for positive cells found in the image (in um²)
- **Positive Nuclei W1 Integrated Intensity:** The total pixel intensity of the nuclear stain over the nuclear area in positive cells
- **Positive Nuclei W1 Average Intensity:** The average pixel intensity of the nuclear stain over all the nuclear areas in positive cells
- Positive Nuclei W2 Integrated Intensity: The total pixel intensity of the marker stain over the nuclear area in positive cells
- Positive Nuclei W2 Average Intensity: The average pixel intensity of the marker stain over all the nuclear areas in positive cells



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- Negative Nuclei Total Area: The total area of nucleus for negative cells found in the image (in um²)
- Negative Nuclei Mean Area: The average area of nucleus for negative cells found in the image (in um²)
- Negative Nuclei W1 Integrated Intensity: The total pixel intensity of the nuclear stain over the nuclear area in negative cells
- Negative Nuclei W1 Average Intensity: The average pixel intensity of the nuclear stain over all the nuclear areas in negative cells
- Negative Nuclei W2 Integrated Intensity: The total pixel intensity of the marker stain over the nuclear area in negative cells
- Negative Nuclei W2 Average Intensity: The average pixel intensity of the marker stain over all the nuclear areas in negative cells



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Cell Data (cell-by-cell measurements)



- Cell: Assigned Label # Cell label number (1 through total cell number)
- Cell: Classification "Positive" or "Negative"
- Cell: Nuclear Area Total square microns of the nucleus as defined by the W1 stain
- Cell: Cell Area Total square microns of the cell (nucleus + cytoplasm if cytoplasm selected in the settings)



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Cell Data (cell-by-cell measurements)



- Cell: W1 Integrated Nuclear Intensity – Total pixel intensity of the nuclear stain in the nucleus
- Cell: W1 Average Nuclear Intensity

 Average pixel intensity of the
 nuclear stain in the nucleus
- Cell: W2 Integrated Nuclear Intensity – Total pixel intensity of the W2 stain in the nucleus
- Cell: W2 Average Nuclear Intensity

 Average pixel intensity of the W2 stain in the nucleus



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Cell Data (cell-by-cell measurements)



- Cell: W2 Integrated Cell Intensity Total pixel intensity of the W2 stain in the cell (nucleus + cytoplasm if cytoplasm selected in the settings)
- Cell: W2 Average Cell Intensity Average pixel intensity of the W2 stain in the cell (nucleus + cytoplasm if cytoplasm selected in the settings)



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