# Matrix ORientation and Texture EXplorer (MORTEX)

## Instruction Manual

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## Overview

**MORTEX** is an ImageJ macro for automated processing and feature extraction of digital extracellular matrix (ECM) microscopy images obtained with fluorescence imaging of Picrosirius red-stained tissue sections.

For the latest version, please visit the repository at www.github.com/cjravensbergen/MORTEX. **MORTEX** is available for public use under the terms of the *GNU General Public License (GPL) v3*.

*** Please follow best practice guidelines for image analysis and maintain fixed acquisition and processing parameters during experiments. ***

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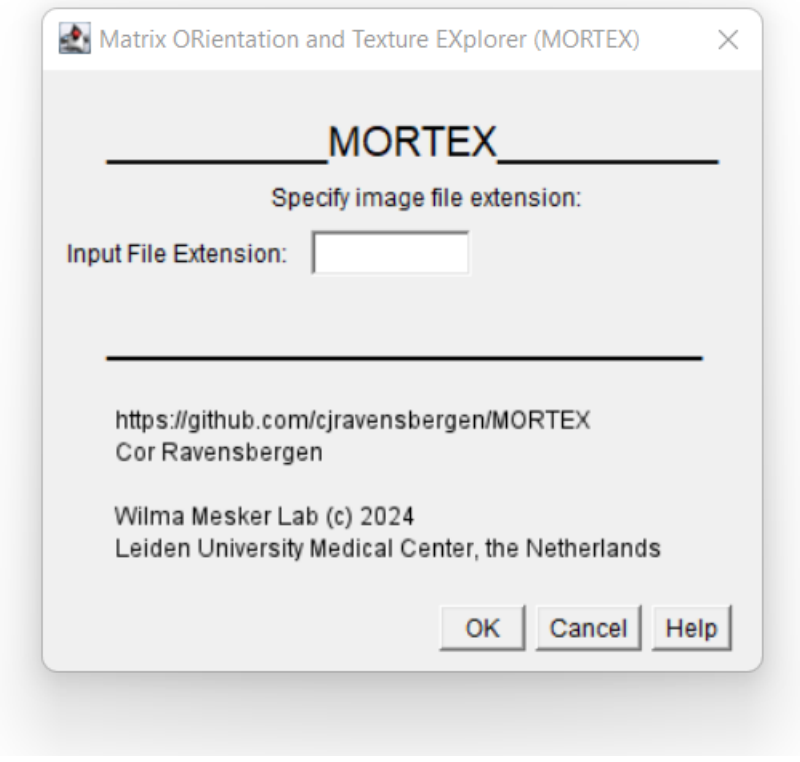
## Interface

**MORTEX** offers a minimalistic user interface for running its analysis module. Upon starting the **MORTEX** ImageJ macro, users are prompted to input the image file extension.




Press `OK` and a new window will pop up, prompting the user to select an image input directory. A second window will pop up prompting the use to select a directory for the output results file (.txt).

**MORTEX** provides quantitative output for ten matrix and fiber parameters (see description table below). The macro is optimized for batch processing of multiple images, returning a dataframe with measurements to the user-specified output directory.

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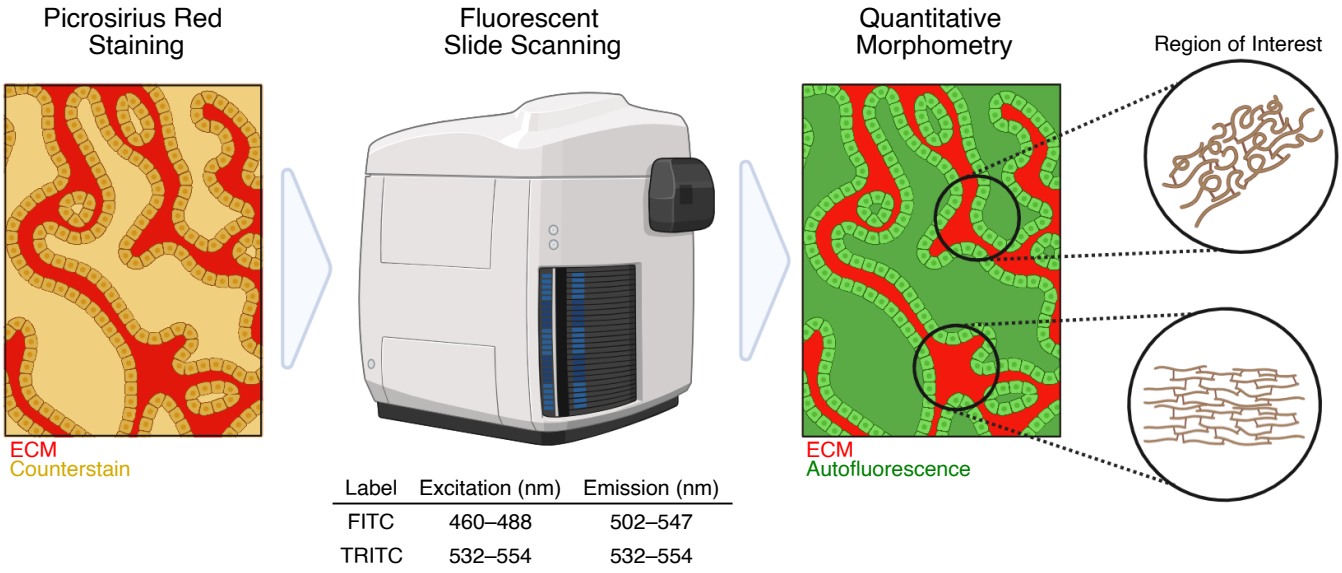
The next sections describe the recommended experimental setup and pre-processing steps of **MORTEX** and provides a description table of the ECM parameters measured.

# Experimental Setup

**MORTEX** is designed to analyze microscopy images obtained from fluorescent imaging of Picrosirius red-stained tissue sections. The experimental setup required to generate input images for **MORTEX** is shown below. Fluorescent acquisition using FITC (for autofluorescence) and TRITC (for ECM visualization) filters is recommended. The use of regions of interest (ROI) is advised to reduce processing times.




| Label | Excitation (nm) | Emission (nm) |
|---|---|---|
| FITC | 460–488 | 502–547 |
| TRITC | 532–554 | 532–554 |

# MORTEX Pre-Processing Steps

**MORTEX** applies basic image pre-processing before image analysis. The sequential pre-processing steps are described below.

*Split Channels*

Multichannel images are split into single channel images, where the ECM fiber image (TRITC signal) is retained for downstream analysis.

*8-bit conversion*

*Thresholding*

Fiber images are thresholded using ImageJ's 'Auto Threshold' function with default method, black and white are ignored.

*Denoising*

Thresholded fiber images are denoised using ImageJ's 'Despeckle' function.

*Skeletonization*

Thresholded fiber images are skeletonized using ImageJ's 'Skeletonize' function.

# ECM Parameter Description Table

| ECM Parameter | Description |
|---|---|
| **Tortuosity** | Measures the complexity or winding nature of fiber paths within the ECM. Higher tortuosity indicates more twisting and turning paths. |
| **Compactness** | The ratio of the area of a fiber network to its perimeter, indicating the compactness or spread of the structure. A high ratio suggest a compact ECM. |
| **Uniformity** | Measures the texture of the ECM by analyzing similarity in pixel intensities of neighboring fibers. High uniformity indicates a uniform matrix with repetitive elements. |
| **Intersection density** | Quantifies the number of intersections or cross-points between fibers within a defined area, indicating the degree of connectivity and complexity of the network. |
| **Fractal dimension** | A measure of the ECM's complexity and how detail changes with scale. Higher values indicate more intricate, space-filling patterns that are self-similar across scales. |
| **Fiber bundle density** | Represents the concentration of fiber bundles in a given area, indicating the thickness and robustness of the ECM network. Higher density suggests more tightly packed fibers. |
| **Dominant direction** | Indicates the primary orientation or alignment of fibers within the ECM, represented as an angle. It shows the main structural direction of the ECM. |
| **Branching density** | Measures the number of branching points within the ECM, indicating the extent of network branching. High branching density suggests a complex, tree-like network structure. |
| **Average fiber length** | The mean length of individual fibers in the ECM. Longer fibers indicate elongated structures, while shorter fibers suggest more fragmented or crosslinked configurations. |
| **Anisotropy index** | Measures the degree of directional dependence in the ECM structure, showing how much the fibers are oriented in a particular direction. Higher anisotropy indicates more alignment. |

# Contact & Contributions

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[PUBMED link]

## Dependencies

- *Bio-Formats Importer*
- *GLCM2, version 1.0.1 (https://github.com/miura/GLCM2)*
- *OrientationJ, version 2.0.5 (https://bigwww.epfl.ch/demo/orientation/)*
- *AnalyzeSkeleton*