Instructions for using code developed in “Robust collagen texture quantification in nonlinear microscopy by combining the gradient structure tensor with a mixed noise model”.

Programming platform: Matlab R2024b.

I Synthetic pattern generation

Prompt:

[I,GenerativeField,SIGParameters]=syntheticImageGeneration;

to generate an image stack I containing fibrous pattern with directions dictated by the 2D vectorial field GenerativeField and generative parameters defined in the fields of the structure SIGParameters.

Image size, number of slices and other settings are set by input dialog windows: one to choose from a presets of pattern, one for general image properties, one for directional field properties, and one for fiber drawing settings.

To input the settings directly, prompt:

[I,GenerativeField,SIGParameters]=syntheticImageGeneration(SIGParameters,Size,SliceNumber,Spacing,Class,SIGParametersIn,flag\_figure);

where:

SIGParameters:

- structure defining all the parameters for image stack synthesis or,

- char defining the name from presets ('I Straight Lines', 'II Coherent Waves', 'III Uncoherent Waves', 'IV Coherent Wavelets', 'V Uncoherent Wavelets', 'VI Entangled','VII Chaotic', 'VIII Composite', 'IX Multiscale Waves', 'X Fuzzy Multiscale Waves', 'Braids', 'Whirlpools', 'Nodes', 'Curls').

Size: 1 x 2 vector,size of each slice.

SliceNumber: number of slices in the stack.

Spacing: 1 x 3 vector with pixel spacing along x and y and slice spacing along z, define spatial units for processing, default is [0.34 0.34 1].

Class: char defining the numerical class of output,

'uint16' (unsigned 16 bit integer, default) or 'double' (64 bit floating point integer) .

SIGParametersIn: structure with a subset of custom parameters to overwrite the preset.

flag\_figure: binary flag for showing the resulting image as montage.

**Example 1.1)** to generate an uint16 image stack of 512x512x10 with pattern 'III Uncoherent Waves', spacing [0.5 0.5 2], a certain distribution of noise and background and create a figure:

clear SIGParametersIn % to clear previous settings if present

SIGParametersIn.AverageSignal=1000;

SIGParametersIn.SNRShot=20;

SIGParametersIn.SNRMult=20;

SIGParametersIn.SNRWhite=20;

SIGParametersIn.SDR=10;

SIGParametersIn.TissuePercentage=40;

SIGParametersIn.BackgroundPercentage=20;

SIGParametersIn.BackgroundSigma=20;

[I,GenerativeField,SIGParameters]=syntheticImageGeneration('III Uncoherent Waves',[512 512],10,[0.5 0.5 2],[],SIGParametersIn, true);

**Example 1.2)** to generate a double precision image stack of a clean pattern (without noise or background):

clear SIGParametersIn % to clear previous settings if present

SIGParametersIn.SNRShot=inf;

SIGParametersIn.SNRMult=inf;

SIGParametersIn.SNRWhite=inf;

SIGParametersIn.TissuePercentage=100;

SIGParametersIn.BackgroundPercentage=0;

SIGParametersIn.NoiseEstimateBypass='on';

[I,GenerativeField,SIGParameters]=syntheticImageGeneration('I Straight Lines',[512 512],10,[0.5 0.5 2],'double',SIGParametersIn, true);

The last parameter NoiseEstimateBypass is set ‘on’ to skip the noise estimation in the following pattern analysis (since there is no background) and directly use the input values. This comes useful when characterizing a pure pattern.

Correspondence of pattern names between article and code [article = code]:

I. Straight Lines = 'I Straight Lines',

II. Waves = 'III Uncoherent Waves'

III. Multiscale Waves = 'IX Multiscale Waves'

IV. Wavelets = 'V Uncoherent Wavelets'

V. Incoherent waves = 'VI Entangled'

VI. Chaotic Fibers = 'VII Chaotic'

VII. Realistic Fibers = 'X Fuzzy Multiscale Waves'

Image generation parameters and their relation with article:

|  |  |  |
| --- | --- | --- |
| Parameter name in code | Parameter name in manuscript | Description |
| Size |  | Size of each slice of the image stack. |
| SliceNumber |  | Number of slices in the image stack. |
| Spacing |  | [1 x 3] Spacing between voxels in the first, second, and third dimension. |
| Class |  | Numerical precision of the image output, like ‘double’ or ‘uint16’ (unsigned integer 16 bit). |
| HRGeneration |  | ‘on’ or ‘off’. Trigger generation of image at twice resolution and downscaling. |
| TissuePercentage |  | Percentage of area at full signal intensity in each slice. |
| BackgroundPercentage |  | Percentage of area at zero signal intensity in each slice. |
| BackgroundSigma |  | Sigma for Gaussian smoothing of modulation mask. Define the scale of distribution of background in the image. |
| AverageSignalDefinition |  | How the average signal at full intensity is defined. ‘mean’ or number for percentile. |
| AverageSignal |  | Average signal at full intensity (1000 in the manuscript). |
| SNRShot |  | SNR of synthetic shot noise at full image intensity. |
| SNRMult |  | SNR of synthetic multiplicative noise at full image intensity. |
| SNRWhite |  | SNR of synthetic baseline white noise at full image intensity. |
| SDR |  | Signal to dark current ratio. Defines the baseline (dark current) to add to the image as AverageSignal/ SDR. |
| NoiseEstimateBypass |  | ‘on’ or ‘off’. If on the noise coefficient estimation is bypassed by the generative coefficient during analysis. To be used for clean images without background. |
| SinWave |  | Formulation of the perturbation field, mixing between Eq. 21 (SinWave=0) and Eq. 23 (Sinwave=1). |
| Wavelength | **(μm)** | Wavelength for sine in Eq. 23. |
| AngleSigma | **(μm)** | Scale of pattern variation (sigma of low pass filter). |
| AngleHighSigma | **(μm)** | Scale of pattern variation (sigma of high pass filter). |
| AngleMean | **(deg)** | A direct number or ‘progressive’ for spanning the half circle through the slices of the stack. |
| AngleSD | **(deg)** | Circular standard deviation of directional field angles (spread of directions). |
| AngleJitter |  | Sigma (deg) for random (normally distributed) angular shift for fibers, to introduce chaos in the pattern. |
| AngleSpatialJitter | **(μm)** | Sigma for random (normally distributed) spatial shift for fibers, to introduce chaos in the pattern. |
| AngleRangeLimiter |  | ‘exp’ or ‘none’. Avoid in the directional field circular loops or curls in the fibers. |
| FieldCoalescence |  | Rotate the angle of the directional field by FieldCoalescence\*90 deg. Combined with AngleRangeLimiter  ‘none’, the fibers transition from circular to spiral converging patterns. |
| AngleFieldOctaves | **(au)** | Weights of the directional field multiscale approach in Eq. 24. The input order is inverted. |
| OctaveIntensity | **(au)** | Weights of the multiscale drawing approach in Eq. 26. |
| SeedMode |  | 'random' for randomly spaced seeds (starting points of fibers) or ‘spaced’ for spacing the seeds at (almost) regular intervals. |
| SeedAvoidance |  | 'true'. |
| SeedSpacing |  | When SeedMode is ‘spaced’, spacing between seeds. |
| SeedRandomization |  | When SeedMode is ‘spaced’, randomly shift seeds off-center by a fraction of SeedSpacing. |
| FiberNumber |  | Number of fibers or set to ‘auto’ to fill in the image with fibers automatically according to FiberDensity. |
| FiberDensity |  | ‘loose’ place seeds 1 px apart from preexisting fibers, ‘packed’ place seed 0 px apart, [scalar] set FiberNumber= FiberDensity  \*(number of pixel in slice)/ FiberLength. |
| FiberLength | **(px)** | Length of fibers in slice. If a vector multiple lengths are used in proportions according to FiberLengthIntensity. |
| FiberFading |  | Tapering shape for fiber intensity, ‘sin’, ‘circle’, or ‘none’. |
| FiberBlendingMode |  | Method of blending the new fiber image in the image . If ‘add’ is (I^n+B^n)^(1/n), where n= FiberBlendingExponent, with n=2 follows Eq. 25.  If ‘max’ use the maximum. If ‘exp’ use a negative exponential approach of the type 1-exp(-(I+B)\*fact). |
| FiberBlendingExponent |  | Exponent for fiber blending when FiberBlendingMode  = ‘add’. (I^n+B^n)^(1/n) where n= FiberBlendingExponent. |
| FiberLengthDistribution |  | If fiber length is distributed around its mean value according to a distribution. ‘none’, 'Poisson', 'Maxwell', 'Chi4', 'Chi5' |
| FiberLengthIntensity |  | Weights for combining multiple fiber lengths. |
| FiberLengthRescale |  | ‘on’, ‘off’. When drawing fibers with multiscale approach, rescale the desired fiber length (in px) according to the active scale, so that only the thickness of the fiber is affected in the final result. |

II Pattern analysis

Prompt:

[TImageProperties,TAnalysisSetting,TNoise,TGradient,TGradientHisto,TStructure,TStructureHisto]=patternAnalysis(I,Spacing);

where Spacing is a 1 x 3 vector specifying voxel spacing (if I is synthetic, it is retrieved from SIGParameters.Spacing), to perform the blind analysis on the image stack I and outputting the results in tables. Analysis settings are given in dialog windows for noise estimation, background detection, and general structure tensor analysis. Image properties are stored in table TImageProperties and the processing settings are stored in table TAnalysisSetting. The other tables present results per slice and their average and SD. An option to store the tables in the separate sheets of an xlsx file is offered.

To input the setting directly (without dialog windows) for blind analysis, prompt:

[TImageProperties,TAnalysisSetting,TNoise,TGradient,TGradientHisto,TStructure,TStructureHisto]=patternAnalysis(I,Spacing,NEParametersIn,BDParametersIn,STParametersIn,[],FigureSliceNumber,FlagSave);

where NEParametersIn, BDParametersIn, STParametersIn are structures with fields defining the parameters for noise estimation, background detection, and general structure tensor analysis, respectively, FigureSliceNumber is the index of a slice to show as example in a figure (if different from 0), and FlagSave is a logical for saving the results to an xlsx file.

To perform a non-blind analysis, inputting also the parameters obtained from image generation (SIGParameters and GenerativeField) and validate noise estimation and angular index, prompt (changes highlighted):

[TImageProperties,TAnalysisSetting,TNoise,TGradient,TGradientHisto,TStructure,TStructureHisto, TSyntheticAngle, TGenerativeField]=patternAnalysis(I, SIGParameters ,NEParametersIn, BDParametersIn, STParametersIn, GenerativeField ,FigureSliceNumber ,FlagSave);

Note that in this case the second input is the structure containing all the properties of image generation, instead of the simple spacing vector.

The output tables TSyntheticAngle and TGenerativeField contain comparison between theoretical and measured values.

**Example 2.1)** blind analysis of a previously generated synthetic image I (for instance from example 1.1) with dialog windows:

[TImageProperties,TAnalysisSetting,TNoise,TGradient,TGradientHisto,TStructure,TStructureHisto]=patternAnalysis(I, SIGParameters.Spacing);

**Example 2.1)** non-blind analysis of a previously generated syntetic image I with dialog windows:

[TImageProperties,TAnalysisSetting,TNoise,TGradient,TGradientHisto,TStructure,TStructureHisto, TSyntheticAngle, TGenerativeField]=patternAnalysis(I, SIGParameters ,[], [], [], GenerativeField ,3 ,true);

Note that the dialog windows are activated if NEParametersIn, BDParametersIn, STParametersIn are all empty, otherwise changes the custom parameters provided and keep the others to default.

General Description of the Output Tables

|  |  |  |
| --- | --- | --- |
| Table Name (with above prompts) | Sheet Name in File | General Description |
| TImageProperties | Image Properties | Available image properties: size, spacing, and the generative parameters as passes in SIGParameters. |
| TAnalysisSetting | Processing Parameters | A list of the parameters used for analysis. |
| TNoise | Noise Descriptors | Statistics describing the amount of noise and SNR found in the images, separated in foreground and full signal areas. |
| TGradient | Global Structure Descriptors | Signal mean and SD in the foreground. Area percentage of , , and remaining viable area for gradient analysis (blue, cyan, and neutral in the gradient figure). Coherence, DA, eccentricity, and angle obtained from the diagonalization of the global structure tensor |
| TGradientHisto | Gradient Angular Distribution | Results from the analysis of gradient derived directions and their histogram. Different measures for angular average and spread are computed. |
| TStructure | Averaged Local Structure | Area percentage of , low structure SNR, acceptable structure SNR but low anisotropy, high anisotropy (blue, red, neutral, and yellow in the structure tensor figure). Mean and SD (over the slice pixels) of the coherence, DA, eccentricity, and angle obtained from the diagonalization of the local (pixelwise) structure tensor |
| TStructureHisto | Structure Angular Distribution | Results from the analysis of structure derived directions and their histogram. Parallels those in Gradient Angular Distribution. |
| TSyntheticAngle | Generative Angle Descriptors | Discrepancies between measured angular indices and theoretical indices of synthetic images. |
| TGenerativeField | Generative Field Pixelwise RMS Errors | RMS discrepancy between directional angles of the generative field and those detected by analysis. |

Output indices in tables and file.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Index name in manuscript | Table Name (with above prompts) | Sheet Name in File | Column Name | Column Letter |
|  | TStructureHisto | Structure Angular Distribution | Angle Mean | B |
|  | TStructureHisto | Structure Angular Distribution | Angle Std | C |
|  | TStructureHisto | Structure Angular Distribution | Shannon Entropy | H |
|  | TStructure | Averaged Local Structure | Degree of Anisotropy Mean | I |
|  | TGradient | Global Structure Descriptors | Degree of Anisotropy | H |
|  | TGradientHisto | Gradient Angular Distribution | Angle Mean | B |
|  | TGradientHisto | Gradient Angular Distribution | Angle Std | C |
|  | TGradientHisto | Gradient Angular Distribution | Shannon Entropy | H |

Input settings:

Parameters for noise estimation (fields of NEParameters)

|  |  |
| --- | --- |
| Parameter name in code | Description |
| HighpassWavelength | Wavelength (px) of the highpass filter to isolate the high frequency noise in the image. |
| PatchSize | Side size (px) of the overlapped 2D square patches (blocks) for block processing. |
| BinSize | Number of element contained in each bin during binning operations over the blocks. |
| SeparateSlices | flag to perform the noise estimation per slice. Used during validation. |
| VartotCutoffPrc | Cutoff percentile for blocks with higher intensity during estimation. |
| VartotTrimPrc | Cutoff percentile to trim off the binned blocks during estimation, based on block total variance, a two-side trim of the blocks of each bin is performed. |
| VartotLowPrc | Cutoff percentile to select lowest variance as representative of no signal area for estimation. |
| DarkCurrentTrimPrc | Percentile for the trimmed mean used to compute from the no signal blocks. |
| ICutoffPrc | Cutoff percentile for blocks with higher intensity during noise coefficients estimation. |
| VarTrimPrc | Cutoff percentile to trim off the binned blocks during noise coefficients estimation, based on noise variance. |
| ExtraFigureSliceNumber | If SeparateSlices is true, show the noise estimation process details in this slice. |

Parameters for background detection (fields of BDParameters)

|  |  |  |
| --- | --- | --- |
| Parameter name in code | Parameter name in manuscript | Description |
| SignalSmoothingRadius | **(μm)** | Radius of the Gaussian smoothing in the computation of the signal SNR (Eq. 3). |
| Smoothing3D |  | True or false. If the smoothing is activated also along image depth. |
| SNRThreshold |  | Threshold on the signal SNR to produce the background mask. |
| LowSignalThreshold |  | Threshold on low signal intensity to add further areas to the background mask. |
| MaskSmoothingRadius |  | Radius for extra smoothing of the background mask. |
| AreaOpeningThreshold |  | Number of pixel for area opening and closing for cleaning the final background mask. |

Parameters for structure based analysis (fields of STParameters)

|  |  |  |
| --- | --- | --- |
| Parameter name in code | Parameter name in manuscript | Description |
| FigureSliceNumber |  | Index of the exemplative slice that is shown in the analysis figures. If 0 no figure is produced. |
| FlagSave |  | true or false. To save the results in a xlsx file. |
| GradientDiscretization |  | Type of convolutional kernel for the 2D gradient computation. 'Roberts', 'Prewitt', 'Sobel Tap 3', 'Sobel Tap 5', 'Gaussian Tap 5', 'Gaussian Tap 7', 'Sharr Tap 3', 'Sharr Tap 5', 'Farid Tap 3', 'Farid Tap 4', 'Farid Tap 5'. |
| GradientBiasRemoval |  | true or false. Activate the gradient bias correction explained in the manuscript. |
| GradientNormalization |  | true or false. Normalize the gradient before directional analysis (otherwise the gradient norm act as weight for statistical descriptors). |
| StructureTensorRadius | **(μm)** | Radius of the Gaussian smoothing for the structure tensor computation in Eq. 9. |
| StructureTensorSmoothingDirection |  | ‘2D’ if the smoothing of the structure tensor is performed only on slices, ‘3D’ if it is performed also along image depth. |
| StructureBiasRemoval |  | true or false. Activate the structure bias correction explained in the manuscript. |
| SliceNumberInterval |  | 1 x 2 vector, interval of slice indices to analyze. If ‘auto’ the interval is computed according to SNIBackgroundThreshold. |
| SNIBackgroundThreshold |  | Threshold on maximal allowed percentage of background area to compute SliceNumberInterval. |
| GradientSNRThreshold |  | Threshold on gradient SNR, Eq. 8. |
| StructureSNRThreshold |  | Threshold on structure SNR. |
| AnisotropyDescriptor |  | Type of anisotropy measure used for analysis. 'Coherence', ‘DA’, 'Eccentricity'. |
| AnisotropyThreshold |  | Threshold on AnisotropyDescriptor to segment high anisotropy areas. |
| StructureWeightType |  | Type of weights to be used for statistical analysis of structure. 'Variation Difference', 'Total Variation', 'Signal', 'First Variation'. |
| CropSize |  | Number of pixels to remove around the border prior to statistical analysis. |