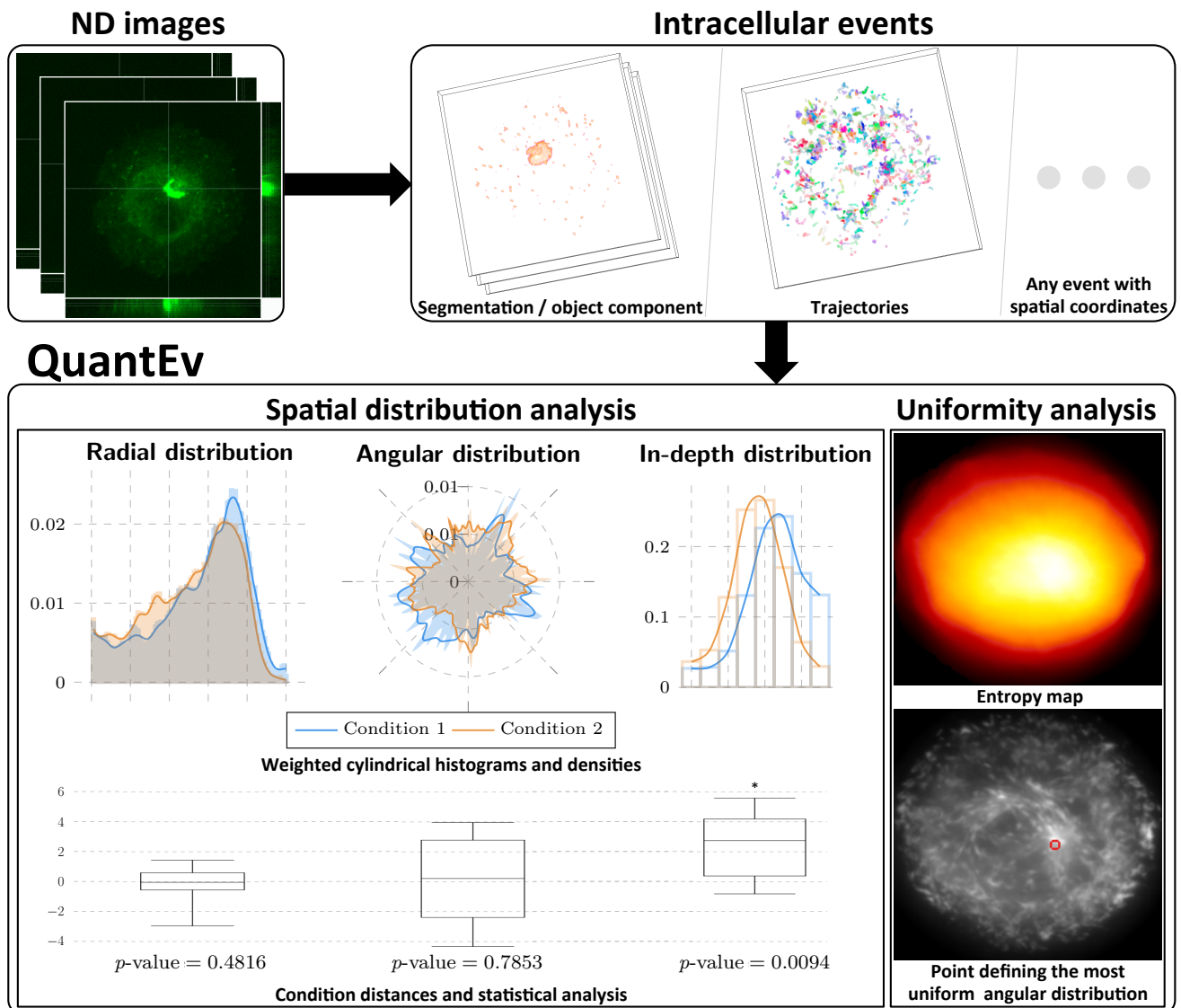


Tutorial for QuantEv Icy plugin



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This document is a tutorial for using **QuantEv** plugin with Icy. QuantEv is a fully automatic and non-parametric framework divided into three distinct operations:

- *QuantEv-Densities*: from an input image (2D, 3D, 2D+t or 3D+t), *QuantEv-Densities* computes **histograms and densities** in the cylindrical, spherical or Cartesian coordinate system,
- *QuantEv-Test*: from an input text file corresponding to densities, *QuantEv-Test* applies a **paired Wilcoxon signed-rank test** between the replicates of each condition,
- *QuantEv-Uniformity*: from an input image (2D, 3D, 2D+t or 3D+t), *QuantEv-Uniformity* finds the reference point in the image corresponding to the **most uniform angular distribution of events**.

To show how to use QuantEv Icy plugin, we propose in this tutorial to apply the different functionalities of QuantEv on **synthetic simulated image sequences**.

1 Introduction to QuantEv modules

1.1 QuantEv-Densities

When you open QuantEv plugin, you obtain the interface shown in Fig. 1.

1.1.1 Inputs

- **Input image**
The input image corresponds to the image for which you want to analyze the spatial distribution. It can be a fluorescence microscopy image in which case you will analyze the intensity distribution. Most of the time, it will be a pre-processed image, *e.g.* segmented particles. This input is mandatory.
- **Channel in input image used for analysis**
This input allows you to choose the channel of the image for which you want to analyze the spatial distribution.
- **Cell mask**
The cell mask enables to take into account the cell shape in the spatial distribution analysis. In this mask, you have to define an intensity superior to 0 in the cell and equal to 0 outside the cell. This input is optional.
- **Exclusion mask**
The exclusion mask allows you to remove a region inside the cell where no event of interest occurs. In this mask, you have to define an intensity superior to 0 in the region in the cell that is excluded from the analysis. This input is optional.
- **Coordinate system for QuantEv**
You can choose a cylindrical, spherical or a Cartesian coordinate system, as well as analyzing the spatial distribution of the events with respect to their distance to the cell border.
- **X coordinate of the system center**
Y coordinate of the system center
Z coordinate of the system center (for spherical coordinate system)
The coordinates of the coordinate system center can be given to QuantEv. If not (-1), the cell center is considered as coordinate system center.

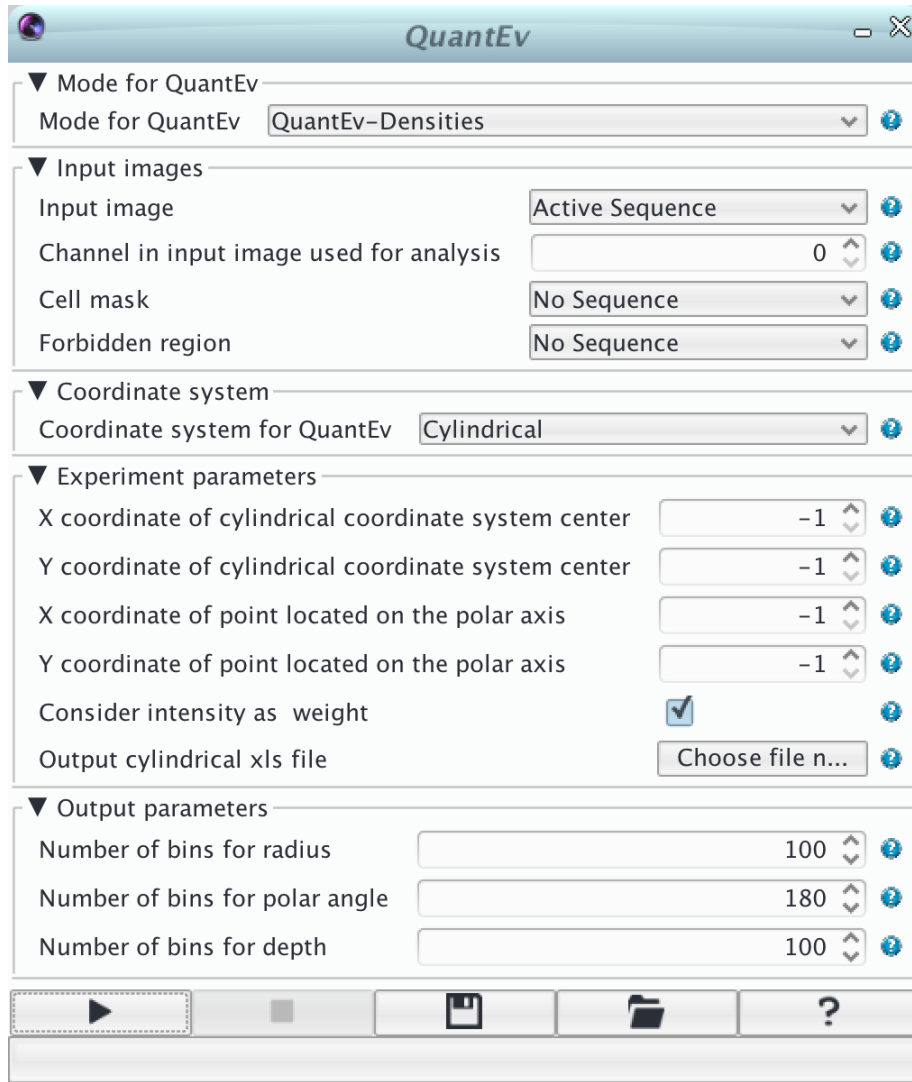


Figure 1: Screenshot of *QuantEv-Densities*.

- X coordinate of a point located on the polar/colatitude/X axis
Y coordinate of a point located on the polar/colatitude/X axis
The polar/azimuthal/X axis is defined by the line between the reference center and the coordinates of a point on this axis. If not given (-1), the horizontal line starting from the reference point to the right is defined as the polar/azimuthal/X axis.
- Consider intensity as weight
The intensity can be taken into account as weight for histograms and densities in the spatial distributions.
- Output xls file
The output xls file name has to be defined to export the results.
- Number of bins for radius/abscissa/distance
Number of bins for polar angle/colatitude/ordinate
Number of bins for depth/azimuth angle
The output specifications allow to define the number of bins for each component.

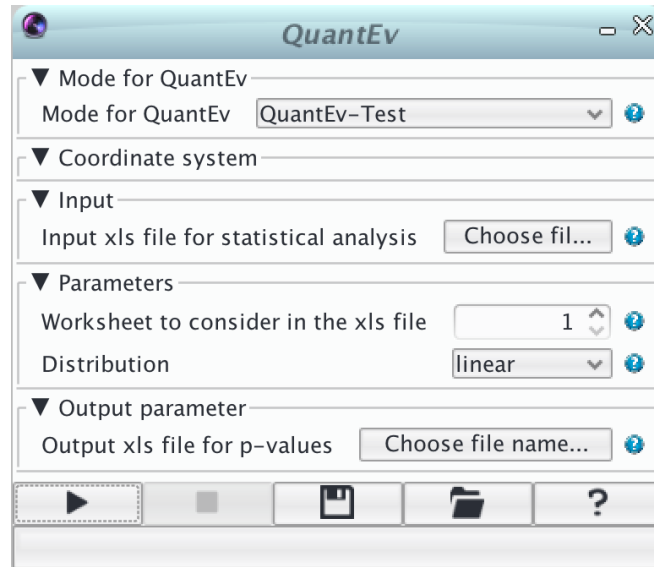


Figure 2: Screenshot of *QuantEv-Test*.

1.1.2 Output

The output of *QuantEv-Densities* is a xls file containing the histograms and the densities. In this file, each worksheet has an explicit name.

1.2 QuantEv-Test

When you select *QuantEv-Test* in the field *Mode for QuantEv*, you obtain the interface shown in Fig. 2.

1.2.1 Inputs

- **Input xls file for statistical analysis**
In this xls file, each column corresponds to one image. The first row of the file contains the different conditions. For a given condition, all the replicated experiments must have the same condition identification.
- **Worksheet to consider in the xls file**
This parameter allows you to select the worksheet in the xls file for which you want to apply a statistical analysis.
- **Distribution**
The distribution is circular for angular distributions and linear for the other distributions.
- **Output xls file**
The output xls file name has to be defined to export the p-values obtained with a paired Wilcoxon signed-rank test for every pair of conditions.

1.2.2 Output

The output xls file contains the p-values for every pair of conditions.

1.3 QuantEv-Uniformity

When you select *QuantEv-Uniformity* in the field *Mode for QuantEv*, you obtain the interface shown in Fig. 3.

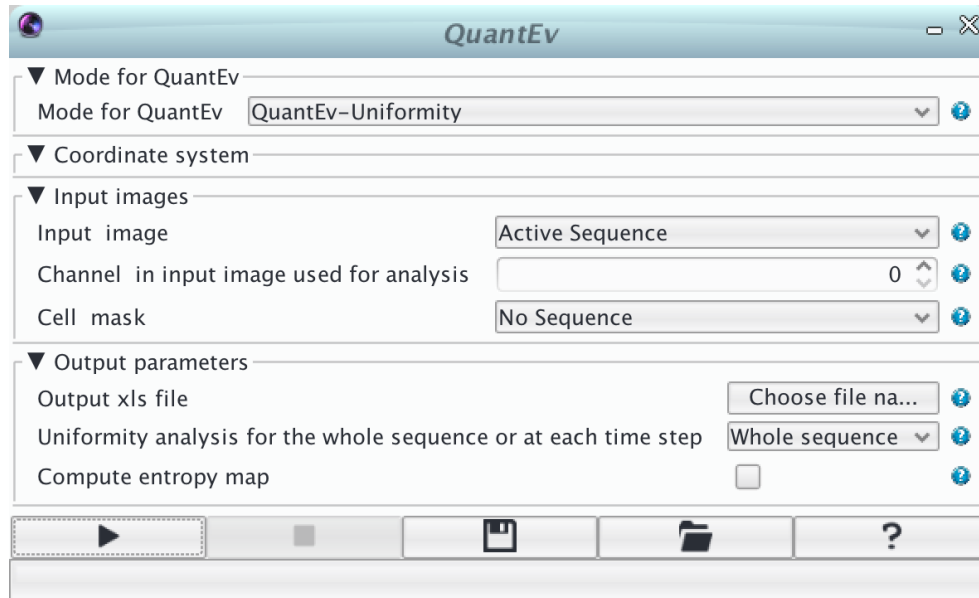


Figure 3: Screenshot of *QuantEv-Uniformity*.

1.3.1 Inputs

- **Input image**
The input image is the image for which you want to estimate the point that gives the most uniform angular distribution. This input is mandatory.
- **Channel in input image used for analysis**
This input allows you to choose the channel of the image for which you want to analyze the spatial distribution.
- **Cell mask**
The cell mask allows to remove the region that is out of the cell from the analysis. In this mask, you have to define an intensity superior to 0 in the cell and equal to 0 outside the cell. This input is optional.
- **Output xls file**
The output xls file name has to be defined to export the coordinates of the point(s) giving the most uniform angular distribution(s).
- **Uniformity analysis for the whole sequence or at each time step**
In case of an image sequence, you can estimate a reference point for the whole sequence or at each time step.
- **Compute entropy map**
You can get an entropy map as the point with the most uniform angular distribution is defined as the reference point with the maximum entropy for angular distribution. However, getting the entropy map will extend computation time as entropy is computed for each point while a bisection method is used otherwise to speed up the computation.

1.3.2 Outputs

The output xls file contains the coordinates of the point that gives the most uniform angular distribution, over the whole sequence or at each time step. The entropy map is also given if required by the user.

2 Test cases

2.1 Cell shape influence

To illustrate the interest of the input cell mask and the region in the cell excluded from the analysis in *QuantEv-Densities*, we propose to analyze the spatial distribution of the same input image sequences

- i) without a cell mask,
- ii) with a cell mask,
- iii) with a cell mask and a region excluded from the analysis.

2.1.1 Data

Let us start with particles uniformly distributed over 16 different paths from the center to the periphery of square shaped-cells (see Fig. 4 first row). 10 simulations are available on the iMANAGE database at <https://cid.curie.fr/iManage/standard/login.html> with username *public* and password *Welcome!1* in the project entitled QuantEv-Data (squaredShapedCell_uniformDistribution_01.tif to squaredShapedCell_uniformDistribution_10.tif). The input cell mask and the region in the cell excluded from the analysis are also available on the iMANAGE database (squareShapedCellMask.pgm and squareShapedCell_regionExcludedFromTheAnalysis.pgm).

2.1.2 Step-by-step analysis

- 1 Select *QuantEv-Densities* in the field *Mode for QuantEv*
- 2 Open the image sequence entitled squaredShapedCell_uniformDistribution_01.tif
- 3 Select squaredShapedCell_uniformDistribution_01.tif as Input image
- 4 Change the number of bins for polar angle to 16 as only 16 paths around the reference center are used by the particles in the simulations
- 5 Select an output file name in the field *Output cylindrical xls file*
- 6 Push the play button to start the analysis
- 7 Repeat steps 2;3;6 for the 9 other image sequences (squaredShapedCell_uniformDistribution_02.tif to squaredShapedCell_uniformDistribution_10.tif)
- 8 Open the image entitled squareShapedCellMask.pgm
- 9 Select squareShapedCellMask.pgm in the field *Cell mask*
- 10 Process again the 10 image sequences
- 11 Open the image entitled squareShapedCell_regionExcludedFromTheAnalysis.pgm
- 12 Select squareShapedCell_regionExcludedFromTheAnalysis.pgm in the field *Exclusion mask*
- 13 Process again the 10 image sequences

Note 1 The particle emitter in these simulations is located in the image center, so the reference center coordinates do not have to be specified

Note 2 The simulations are oriented with image, so there is no need to define a point located on the polar axis

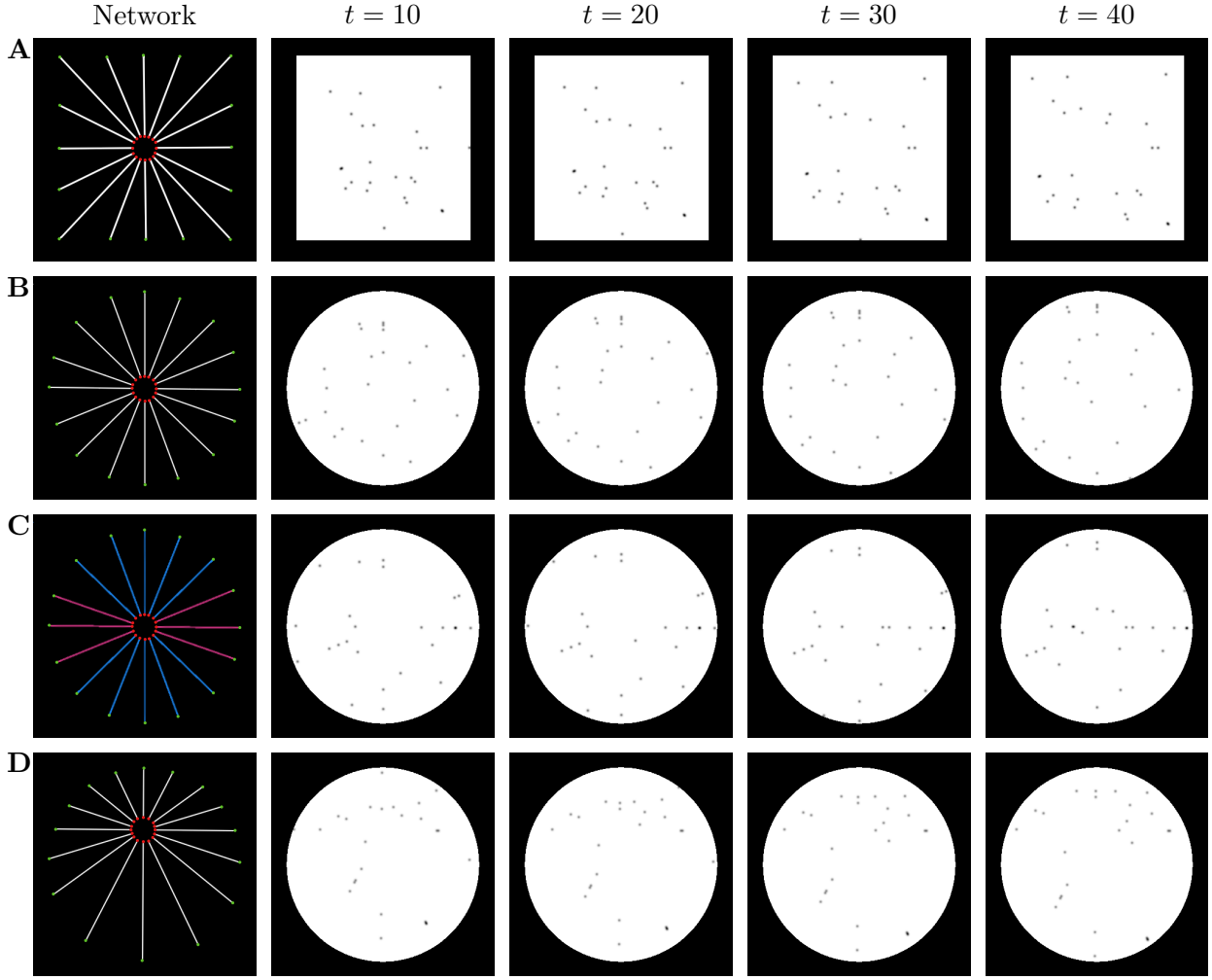


Figure 4: **Synthetic image sequences.** First column: networks used to generate 4 image sequences according to [1]. The particle origins are labeled as red disks while particle destinations appear as green disks. Particles for the sequences A, C and D are uniformly distributed over the different paths. Particles for the sequence C are distributed with a probability equal to 0.1 over the pink paths and with a probability equal to 0.04 over the blue paths. Images corresponding to time $t = 10$, $t = 20$, $t = 30$ and $t = 40$ taken from one simulated image sequence for each network are illustrated in columns 2 to 5.

Note 3 As only the particles are appearing on these simulations, no preprocessing is needed. It is preferable to take intensity into account as several particles can overlap and in that case, their intensity is summed up in the images

Note 4 As the output xls file is not modified over the analysis, the results are put together in the same file, each column corresponding to each processing. The name of the input image is written in the first row of each column

14 For each worksheet in the output xls file, compute the average distribution without cell mask (first 10 analyses), with cell mask (next 10 analyses) and with cell mask and excluded region (last 10 analyses)

15 Use graphs in Excel or OpenOffice to visualize the 3 different analyses

Note 5 The output xls file is available on the iMANAGE database as `testCase1.xls`, attached to the image entitled **Tutorial results**

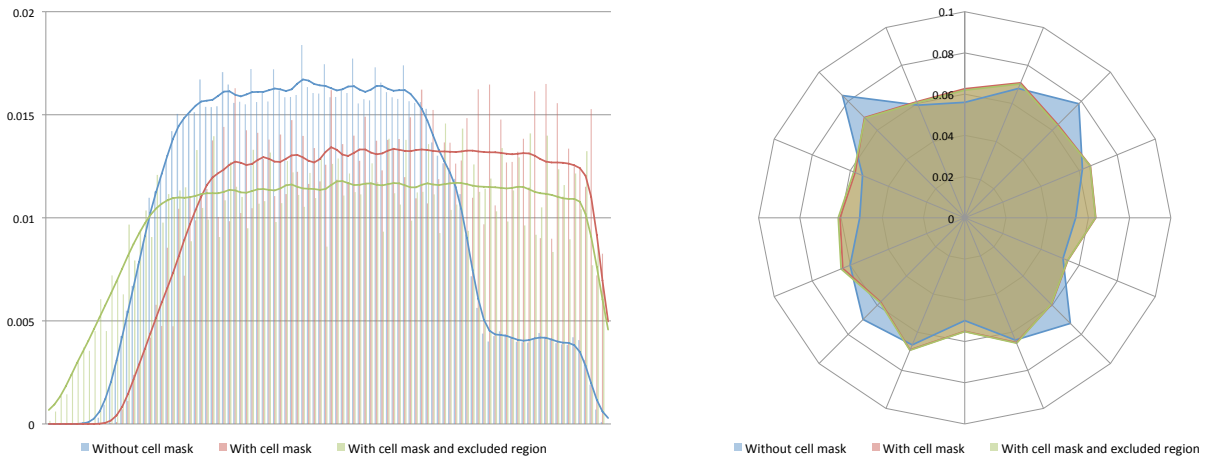


Figure 5: Radial (left) and angular (right) distributions of simulations with square-shaped cells without cell mask, with a cell mask and with a cell mask and an excluded region from the analysis. The bar plots correspond to histograms and the lines correspond to densities.

Note 6 In this example, we use the spherical coordinate system. Spherical and Cartesian coordinate systems are also available, as well as the analysis of the distribution of the distance between events and the cell border

2.1.3 Results

The radial and angular distributions obtained with *QuantEv-Test* are shown in Fig. 5. Without cell mask, the angular distribution shows a squared distribution and the radial distribution is divided into 2 modes: a first plateau corresponding to a distance for which particles are moving on all 16 paths and a second plateau corresponding to particles that travel a longer journey on the diagonal paths. By considering a cell mask, both radial and angular distributions are more uniform. By removing the central region where no particles are trafficking, the radial distribution shows an even more uniform distribution. If the goal is to analyze the spatial distribution of events with respect to a particular area (here the central zone being the particles emitter), it makes much more sense to use a cell mask. In that case, the results correspond to the distribution of events (and not the localization only).

2.2 Distribution comparison and visualization

In this section, we propose to compare image sequences with different particle distributions.

2.2.1 Data

Let us consider 2 series of 10 image sequences showing particles uniformly distributed over 16 paths on a disk-shaped cell (see Fig. 4 second row) and 10 image sequences showing particles isotropically distributed over 16 paths on a disk-shaped cell (see Fig. 4 third row). These sequences as well as the cell mask and the region excluded from the analysis are available on the iMANAGE database (diskShapedCell_uniformDistribution_centeredEmitter_01.tif to diskShapedCell_uniformDistribution_centeredEmitter_20.tif, diskShapedCell_isotropicDistribution_centeredEmitter_01.tif to diskShapedCell_isotropicDistribution_centeredEmitter_10.tif, diskShapedCellMask.pgm and diskShapedCell_regionExcludedFromTheAnalysis.pgm).

2.2.2 Step-by-step analysis

- 1 Select *QuantEv-Densities* in the field *Mode for QuantEv*
- 2 Open diskShapedCellMask.pgm and select it in field *Cell mask*

- 3 Open `diskShapedCell_regionExcludedFromTheAnalysis.pgm` and select it in field *Exclusion mask*
- 4 Process the 30 image sequences presented in Section 2.2.1
- 5 For each worksheet in the output xls file, compute the average distribution for the first 10 image sequences with uniform angular distribution, the 10 next image sequences with uniform angular distribution and the 10 image sequences with isotropic angular distribution to visualize the different distributions (see Fig. 6)
- 6 Select *QuantEv-Test* in the field *Mode for QuantEv*
- 7 Modify the first columns of the xls file (at least) in the density worksheets so all replicates for a given condition have the same identification. For example, change the first row of the 10 first columns into *Uniform angular distribution 1*, the first row of the 10 next columns into *Uniform angular distribution 2* and the first row of the 10 last columns into *Isotropic angular distribution*
- 8 Select the modified xls file as *Input xls file for statistical analysis*
- 9 Select 3 for *Worksheet to consider in the xls file* to compute the statistical test for radial density
- 10 Select a file for *Output xls file for p-values*. If you select the same file as input, a worksheet will be added with the p-values for the statistical test for each pair of conditions
- 11 Select 4 for *Worksheet to consider in the xls file* to compute the statistical test for polar angle density
- 12 Select *circular* in field *Distribution* as polar angle density is circular
- 13 Select a file for *Output xls file for p-values*. Again, if you select the same file as input, a worksheet will be added with the p-values for the statistical test for each pair of conditions

Note 1 The output xls file is available on the iMANAGE database as `testCase2.xls`, attached to the image entitled *Tutorial results*

2.2.3 Results

The radial and angular distributions obtained with *QuantEv-Test* are shown in Fig. 6. The radial distributions for all 3 series of simulations are similar, confirmed by high *p*-values (0.420, 0.378 and 0.861). But the angular distributions of uniformly distributed particles around the cell center are significantly different when compared to the angular distribution of particles isotropically distributed around the cell center ($p\text{-value} = 9.54 \times 10^{-07}$ and $p\text{-value} = 9.54 \times 10^{-07}$). That is not the case when comparing the two series of sequences showing particles uniformly distributed around the cell center ($p\text{-value} = 0.899$).

2.3 Uniformity analysis

In this section, we show how to use *QuantEv* to find the point that gives the most uniform angular distribution in the cell. This can be useful when events are uniformly distributed around a region of interest in the cell, *e.g.* a cell compartment.

2.3.1 Data

We propose to process synthetic images for which particles are uniformly distributed around a region that is not the cell center (see Fig. 4 forth row). 10 image sequences are available on the iMANAGE database (`diskShapedCell_uniformDistribution_uncenteredEmitter_01.tif` to `diskShapedCell_uniformDistribution_uncenteredEmitter_10.tif`).

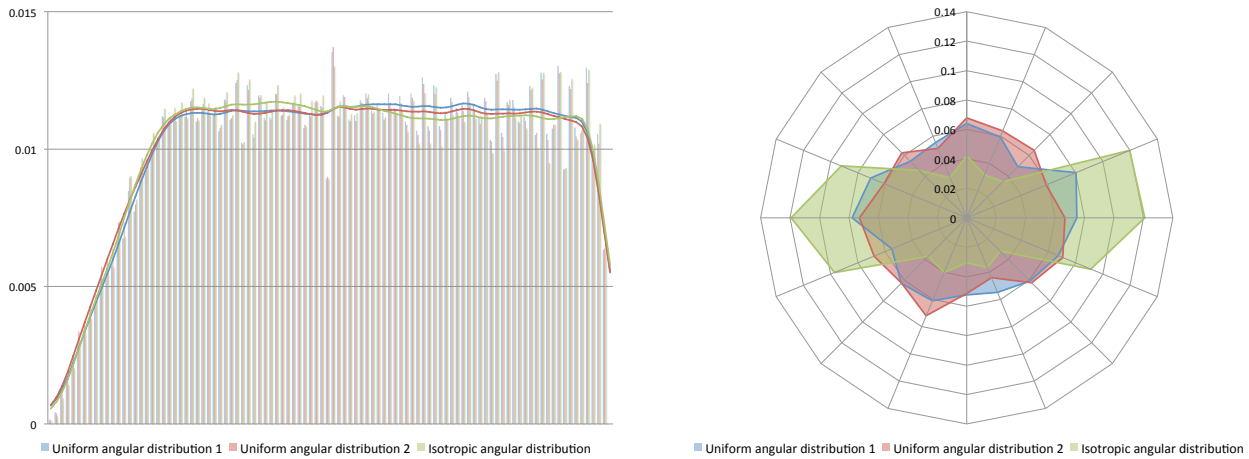


Figure 6: Radial (left) and angular (right) distributions of simulations with disk-shaped cells. The bar plots correspond to histograms and the lines correspond to densities.

2.3.2 Step-by-step analysis

- 1 Select *QuantEv-Uniformity* in the field *Mode for QuantEv*
- 2 Open *diskShapedCell_uniformDistribution_uncenteredEmitter_01.tif* and select it in field *Input image*
- 3 Open *diskShapedCellMask.pgm* and select it in field *Cell mask*
- 4 Select an output file name in the field *Output xls file*
- 5 Push the play button to run the analysis

Note 1 Choosing *Each time step* in the field *Uniformity analysis for the whole sequence or at each time step* computes the point that gives the most uniform angular distribution at each time step

Note 2 Ticking *Compute entropy map* outputs a map with the entropy of the angular distribution at each point (and each time step if *Each time step* is chosen). This processing is longer as it computes angular distribution at each point while a bisection method is used otherwise

Note 3 If the user selects the same output xls file for several analyses, a worksheet will be added for each analysis

Note 4 An xls file with the coordinates of the points that give the most uniform angular distribution for the 10 image sequences is available on the iMANAGE database as *testCase3.xls*, attached to the image entitled *Tutorial results*

2.3.3 Results

The coordinates of the point giving the most uniform angular distribution for each image sequence are close to the actual particle emitter (190,130).

References

- [1] Boulanger J, Kervrann C, Bouthemy P (2009) A simulation and estimation framework for intracellular dynamics and trafficking in video-microscopy and fluorescence imagery. *Med. Image Anal.* 13(1):132–142.