Acapella
Texture Analysis Package

Texture analysis for the Opera system

Sample Application:
Mitochondria Classification

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Texture Analysis with Acapella

Texture analysis is an additional method that can be used to quantify biological effects. It makes use of the “pattern” or structure within the fluorescent region, rather than using the average intensity or shape of the region (see Figure 1-1).

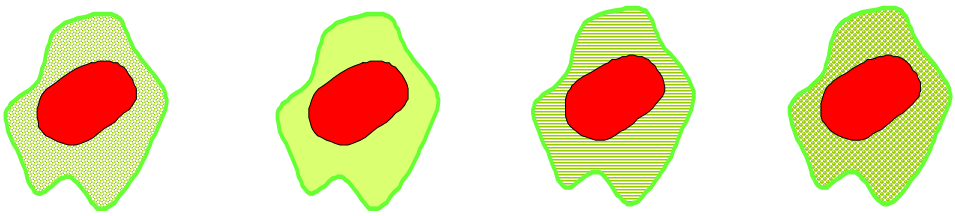


Figure 1-1: Examples of different textures. All cells have the same morphology (shape) and intensities but different textures within the cytoplasmic area.

1.1 Purpose

For an example of a working analysis sequence, see Section 2.

- Texture analysis is performed when quantifying properties of the regions of interest identified in the preceding steps of the analysis sequence.
- In general, texture analysis can be used for two purposes:
  - Image segmentation, i.e. identifying objects or image regions based on their texture. For example, identification of areas covered by cell colonies on bright field images of unstained cells. The cell colonies have a different texture to the background.
  - Object characterization, for example classification of nuclei into apoptotic and healthy nuclei.
- This package provides modules for both purposes.
- Potential application examples are (see Figure 1-2):
  - Evaluation of cytoskeletal structure, e.g. well organized fibres in similar directions versus irregular structures.
  - Nuclear fragmentation, Apoptosis detection.
  - Mitochondrial structure analysis.

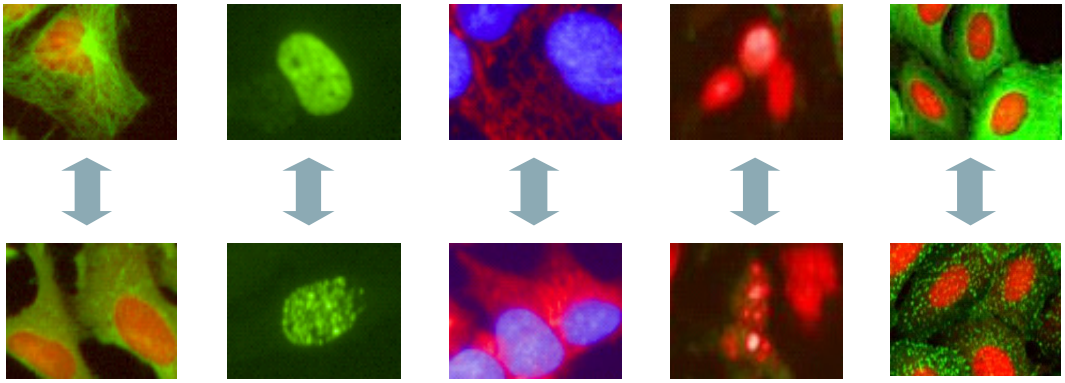


Figure 1-2: Examples of texture changes that can be quantified using using Acapella. From left to right: (a) Tubulin structure change due to Demecolcine treatment. (b) Textures of EdU-stained nuclei. (c) Cytotoxic effects of FCCP on MitoTracker™-Red-stained mitochondria. (d) Nuclear fragmentation of cells stained with Hoechst 33342 (red) and Caspase-3 marker (green). (e) Example images for negative (low) and positive (high) B2 adrenergic receptor activation; in the negative image the green GFP fluorescence is more homogeneous, whereas a strong granularity is visible in the positive image.

- Cytosolic marker structure analysis (smoothness, pits, vesicles), e.g. endosomes, clathrin-coated pits, cellular vesicles, protein aggregates or clusters, homogeneous versus inhomogeneous distribution.
- Nuclear marker structure analysis, e.g. cell cycle proteins, histones, homogeneous versus inhomogeneous distribution.

➤ Typical descriptions associated with texture properties are smooth, rough, granular, spotty, homogeneous, inhomogeneous, irregular, regular, fiber structure, line pattern, etc. If you can describe a phenotype by these terms, try using texture parameters for quantification.

1.2 Background

➤ Because texture recognition is an essential part of our visual perception, it has already been studied for decades from different perspectives, such as human vision, object recognition, machine vision, image segmentation, texture simulation, object tracking, etc.

➤ Due to its complexity and the wide scope of applications, there is no generic solution or system for texture analysis. There are still a number of rather distinct fields of research related to texture, each with many publications every year.

➤ Typically, object classification consists of two steps:

1. Calculation of a set of numerical properties that describe the texture of an object. There is a plethora of different sets of numbers, based on different mathematical approaches. These are either generic or are model-based and tailored to different application areas.
2. Classification of the objects by their set of texture property values using different approaches such as the Fisher Linear Discriminant Analysis (LDA), Support Vector Machines (SVM), Artificial Neural Networks (ANN), etc. Classification is either supervised (i.e. examples of classes are provided by the user) or unsupervised (i.e. different classes are generated by the computer).

➤ This package offers a selection of four different methods for the first step described above (calculating a set of numerical properties to quantify the texture of an object).

➤ These methods have been carefully selected from the many potential methods described in the literature in order to:

- Cover a large variety of different applications for cell image analysis.
- Include diverse mathematical approaches to allow you to select the most suitable.
- Be suitable for an easy-to-use, interactive, visual set-up and tuning (no need for neuronal networks, etc.).

1.3 Texture Feature Calculation

Acapella provides modules for the following feature sets:

Gabor Two selected features that are generated by Gabor filtering. These properties are based on the analysis of spatial repetitions in the texture pattern. A number of parameters need to be adjusted to specify the characteristic “wavelength” and “scale” of the features to be identified.

Haralick – A set of four features (correlation, contrast, homogeneity and sum variance) that are based on the comparison of sets of two pixels in order to identify different textures by their typical intensity co-occurrence patterns. These are a subset of the well-known parameters described by Robert Haralick et al. (1973).

SER Spots, Edges and Ridges – a set of eight properties that are sensitive to different characteristic intensity patterns as described by the property name. These include spots, holes, edges, ridges, valleys, etc. **We recommended this as the first choice for trial and error** as it works well for most applications (see Figure 1-3).

TAS Threshold Adjacency Statistics have been introduced by Nicholas Hamilton et al. (2007). Using a lower and an upper threshold, a mask is calculated representing those pixels that satisfy the given threshold conditions. Thereafter, each pixel is given a score according to the number of its neighbor pixels under the mask. A TAS feature is the relative number of pixels with the given score. TAS features can be calculated very fast.

The modules are named Texture::Calc*Xxx*Features, where *Xxx* is the name of the feature set, e.g. Texture::CalcGaborFeatures.

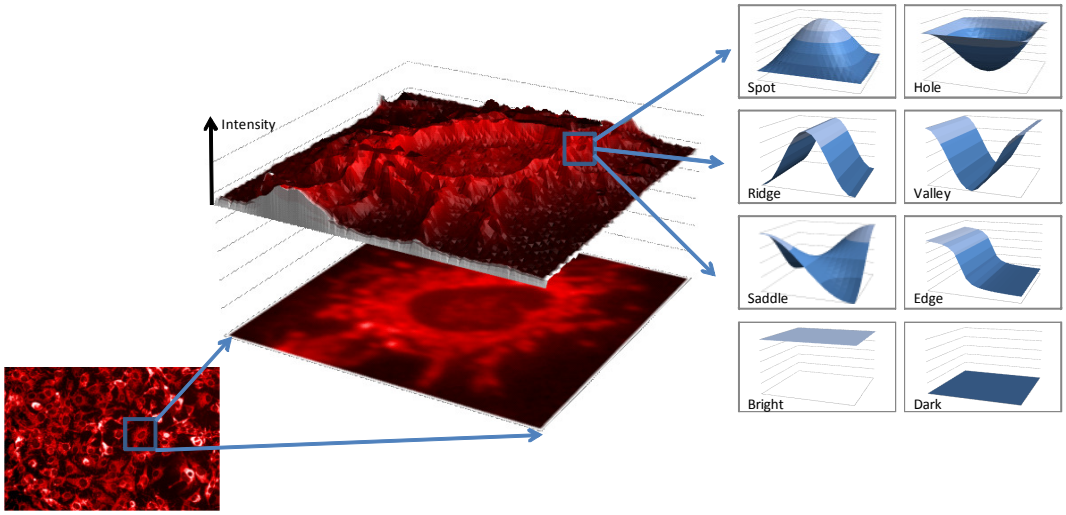


Figure 1-3: Concept of the SER feature set. The intensity structure of a region (here: the cytoplasmic region around a nucleus) is analyzed for the occurrence of typical intensity patterns, e.g. “edges”, “ridges” or “spots”. The biological state is characterized by the frequency of a specific feature or combination of features. *Left:* HepG2 cells with the mitochondria stained with MitoTracker™ Red. *Lower mid*: enlarged region (marked with a blue box). *Upper mid:* pixel intensity depicted as the height of a surface. *Right:* the set of eight SER features identified by the algorithm.

- All Calc*Xxx*Features modules take an objectlist, a stencil from that list and an image as inputs. They quantify for each object the expression of the respective features on the image within the area given by the stencil. A modified objectlist is returned where the feature values are added as attributes. For the feature sets based on image filtering, also filtered versions of the input image are returned, which can be used for segmenting the image by texture.
- The Calc*Xxx*Features modules take optional tuning parameters, e.g., for setting the scale according to the characteristic dimension of the feature being looked for.
- To choose a suitable texture feature for your application, start with the default values. Try several features and select the one which best describes the effect that you are measuring. This is the same principle as applied when selecting the best nuclei and cytoplasm detection methods by trying different algorithms; A, B, C, etc.
- Often the texture of a region can be identified with the visual “pattern” of the intensity distribution, e.g. homogeneous, granular, spotty or stripy. (Our sample assay discussed in Section 2 is of that kind.) Note, however, that some texture features are not perceptible by the pattern recognition of our human eyes and still could provide good assay readouts!

Try using the set of SER Features first. If none of these features give sufficient results then consider testing the feature sets.

- When the best method has been identified you might try tuning the optional parameters.

1.4 Hints and Tips

- The texture features (unless normalization is explicitly disabled) are independent of the overall intensity. This means that cell-to-cell, plate-to-plate or day-to-day variations in the staining intensity have little impact the results. Hence texture features tend to be more robust than absolute intensity or shape-based features.
- Caution! Texture features can be sensitive to image blurring, slightly de-focussed cells and / or imaging of thick structures in different heights. In situations prone to these interferences, e.g. due to the assay setup, cell line, etc., carefully check the robustness with respect to the image acquisition height and consider using intensity or shape-based assay readout values instead of, or in addition to, texture features.
- For a review of texture analysis from the perspective of a computer scientist, see for instance Tuceryan and Jain (1998) or Srinivasan and Shobha (2008).

References

Haralick, R. M., Shanmugam, K., Dinstein, I. (1973) “Texture features for image classification“. IEEE Trans. Systems Man Cybern. **3**:610.

Hamilton, N.A., Pantelic, R.S., Hanson, K., Teasdale, R.D. (2007) “Fast automated cell phenotype image classification”. BMC Bioinformatics **8**:110.

Srinivasan, G. N., Shobha, G. (2008) “Statistical Texture Analysis”. PWASET **36**:1264.

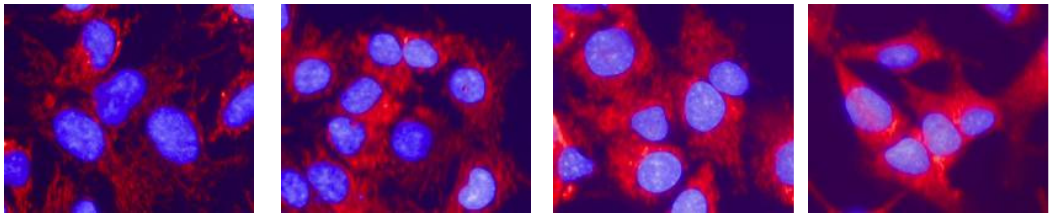
Tuceryan, M., Jain, A. K. (1998) “Texture Analysis”. In: *Handbook of Pattern Recognition and Computer Vision* (2nd Edition) by C.H. Chen, L.F. Pau, P.S.P. Wang (eds.), pp. 207–248, World Scientific Publishing Co.

Sample Script: Mitochondria Classification

This script quantifies biological effects by monitoring changes in the texture of a fluorescent dye. It is taylored to study mitochondria but can be adapted for any other effect that induces textural changes.

2.1 Assay Principle

In the sample assay, nucleus and mitochondria of HepG2 cells have been stained with fluorescent dyes. The addition of a toxic compound induces a change in the texture of the mitochondrial stain (see Figure 2-1): Whereas at low compound concentrations there are dark pits or holes in the otherwise continuous stain, these pits vanish with rising concentration.



Untreated 0.1 µM 3 µM 10 µM

Figure 2-1: Images showing Hoechst 33342 nuclear staining (blue) and MitoTracker™ Deep Red staining (red) of human hepatocellular liver carcinoma (HepG2) cells at different concentrations of a toxic compound (FCCP). As the concentration of the compound is increased (from left to right), there is a notable change in the mitochondrial texture.

2.2 How to Run the Sample Script

- Open the Acapella online help from the Help menu of the top menu bar.
- In the top row, click on “Search” and have it look for “Mitochondria Classification”.
- Browse to the bottom of the document where you find this link box:

Script:	**MitoClassification.script** (click to open in the Player)
Parameters:	**MitoClassification.parameter** (click to load in the Player)
Images:	**Example Images** (click to set in the Player)

- Click on all three links, starting with the top one. This will load the script and its parameters into Acapella and the selects the example images as data source.
- Start the evaluation by click on the green “run” arrow in the toolbar or pressing Ctrl-R.

2.3 General Structure of the Script

The script follows the Opera template for multifield analysis: After getting the images in, the script repeats the same analysis steps for the image pairs (nuclear stain and mitochondrial stain) of all fields, collecting information from all fields in an overall objectlist "allObjects" that holds all cells found in the current well. Then statistical results from this list are returned as output values.

2.4 Image Analysis in Detail

2.4.1 Object Detection

First the nuclear outlines of all cells are determined from the channel with the nuclear stain (nuclei detection). Then the image with the mitochondrial stain is used for finding the overall outline of the cell (cytoplasm detection). Cells that touch the image border are removed from the analysis.

2.4.2 Determination of Intensity and Morphological Properties

The most frequently used shape properties are calculated: cell area and roundness. Then the mean intensity of the mitochondrial stain in the cytoplasmic region is determined and the homogeneity of the intensity, measured as the percentage coefficient of variation (CV) of the intensity in relation to its mean value. For example, a CV of 30% means that the mitochondrial intensity fluctuates by ±30% around the mean value within a cell.

2.4.3 Texture Analysis

The mitochondrial stain is marked by pits or holes that are present in the untreated case but disappear with the addition of a cytotoxic compound. This suggests to employ the *Hole* feature from the SER feature set.

The script calls the module Texture::CalcSERFeatures, which calculates *all* SER features and adds for each of them an attribute SER*Xxx* to the input object list. (Restricting the calculation to just one feature won't spare much computing time as most of the time is needed for image filtering steps common to all SER features.).

Furthermore the module returns the images resulting from the filtering, which can be used for segmenting exactly those regions where the corresponding SER feature is expressed. The script displays the image corresponding to the SERHole feature as an illustration. This image can also be used in optimizing the single tuning parameter of this module, *scale* (cf. Figure 2-2).

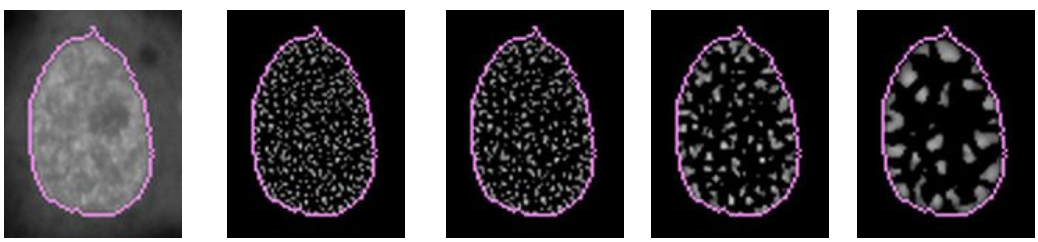


Figure 2-2: Tuning of the *scale* input parameter – here shown for the texture analysis of a nucleus. The leftmost image shows the original marker intensity. All other images are filtered images that are provided by the CalcSERFeatures module. The scale parameter is increased from left to right in four steps and the scale of the structures identified by the image filter changes accordingly. Hence, features of different granularity level can be identified. (That is why we exemplify this with a nucleus image.) To adjust, set the scale parameter to such a value that the filtering detects granules of the proper size, namely those that undergo a change in your assay.

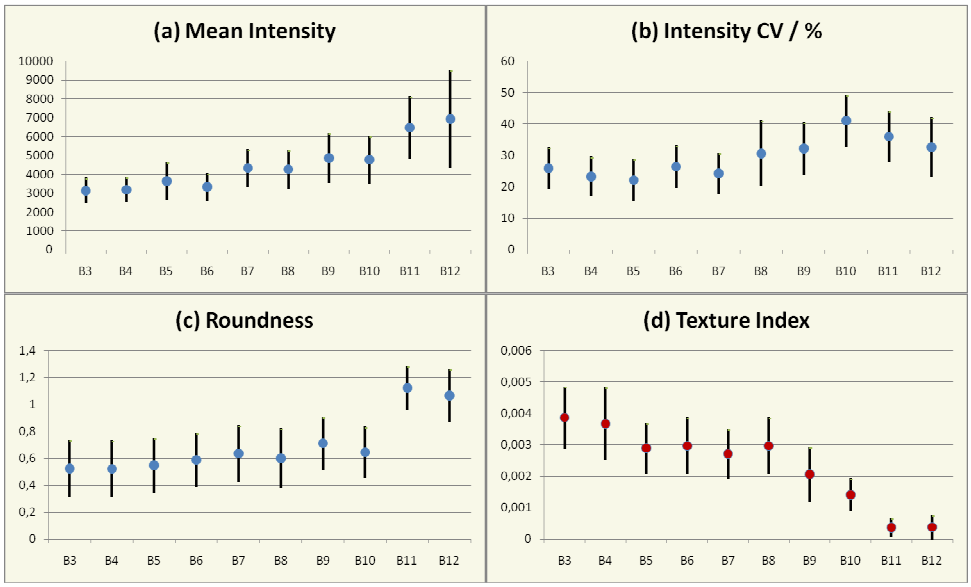
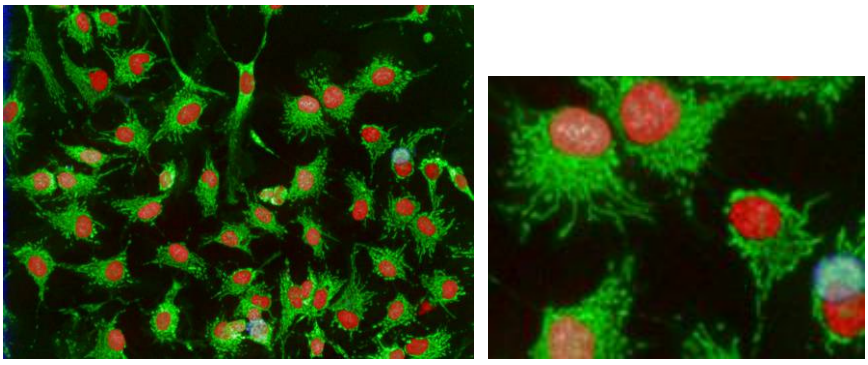


Figure 2-3: Readout values for the sample data set. Images have been taken at a single field per well, each image comprising between 30 and 300 cells. The values are averages over all cells in the image; error bars denote the standard deviation. Each well, B3 through B12, has another concentration of a cytotoxic compound, rising from left to right.

2.5 Results

Figure 2-3 shows the variation of several readouts with rising concentration of a cytotoxic compound. Already with the small statistics of a single image field per concentration value, the texture index obtained by SER hole filtering (d) reliably provides statistically significant effect.

Sample Dataset



Cell type	HepG2
Imaging	20x long WD
Stainings	Hoechst 33342 MitoTracker® Deep Red BOBO™-3
Control Compound(s)	FCCP
Image fields	1 per well
Number of wells	10 (dose-response curve)

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