The state trajectory of cell using Renyi entropy coefficients

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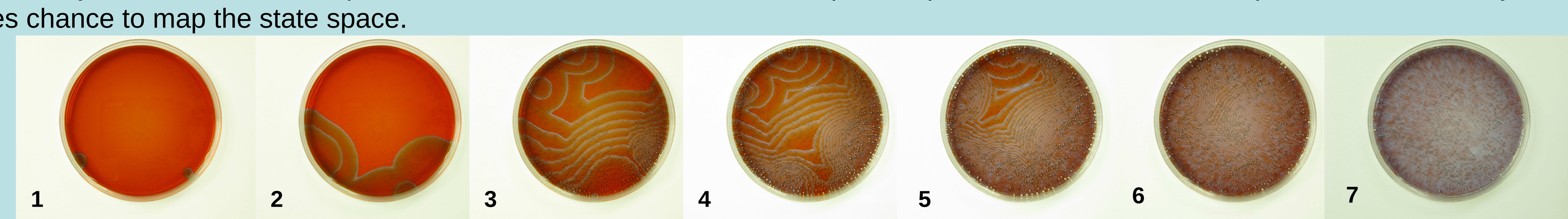
AIM: Cell behavior analysis based on time-lapse microscopy. Objective and automatic construction of the state trajectory of living cell by using only image analysis.

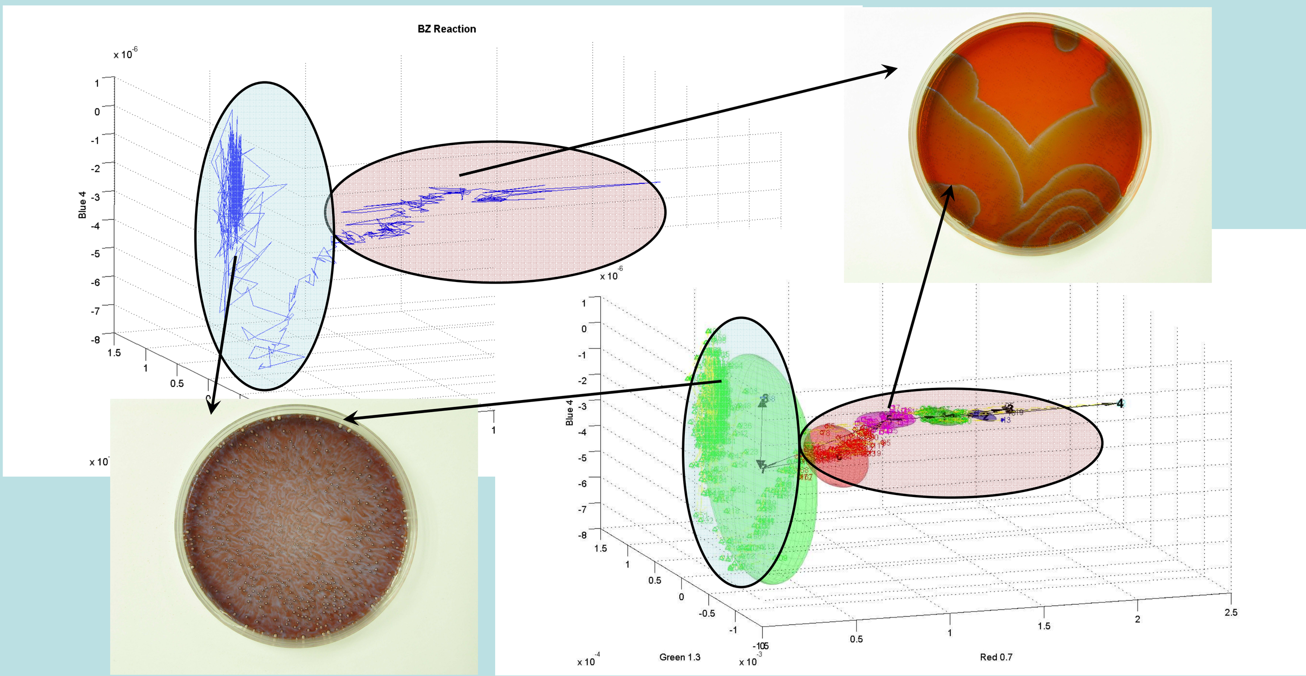
Elementary model of intracellular (or intra-organellar) pattern formation: Belousov – Zhabotinsky reaction in a Petri dish.

The Belousov – Zhabotinsky reaction (Belousov 1958) was devised as a primitive model of citric acid cycle. To the surprise of the authors, it brought about the phenomenon of chemical clock (in mixed systems) or spontaneous pattern formation (in still compartments). We use it as primitive model for testing of our analysis of experiment for the purpose of determination of macroscopic state space.

The distinct feature of the BZ reaction is that although it in fact consists of 80 chemical reactions (György et al. 1990), relatively simple patterns arise and may be modeled by approaches such as cellular automata (numerous demonstrations may be found on the web), ODEs etc.. We decided to search the phase space on the basis of the experiment. Fortunately, the distribution of point numbers between color channels is rather distinct and gives chance to map the state space.



Constructing and clustering of state trajectory

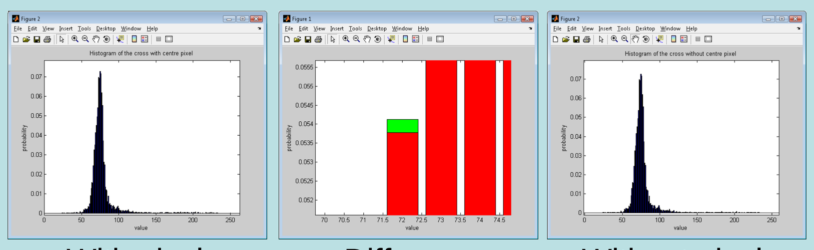


- ❑State coordinates – RGB images → RGB state space
- ❑Expertomica Entropy Calculator → Information Entropy Transformation images
  - ❑ Pseudo colored image – color describes how much information the point carries
  - ❑ Lower alpha parameter enhance rare points
  - ❑ Higher alpha parameter enhance bigger areas
  - ❑ Rényi entropy

$$I_\alpha(X) = \frac{1}{1-\alpha}\log\left(\sum_{i=1}^{n} p_i^\alpha\right)$$

- ❑Point Information Gain (PIG)

$$\Delta I_{x,y,\alpha} = I_\alpha - I_{x,y,\alpha}$$



With pixel | Difference | Without pixel

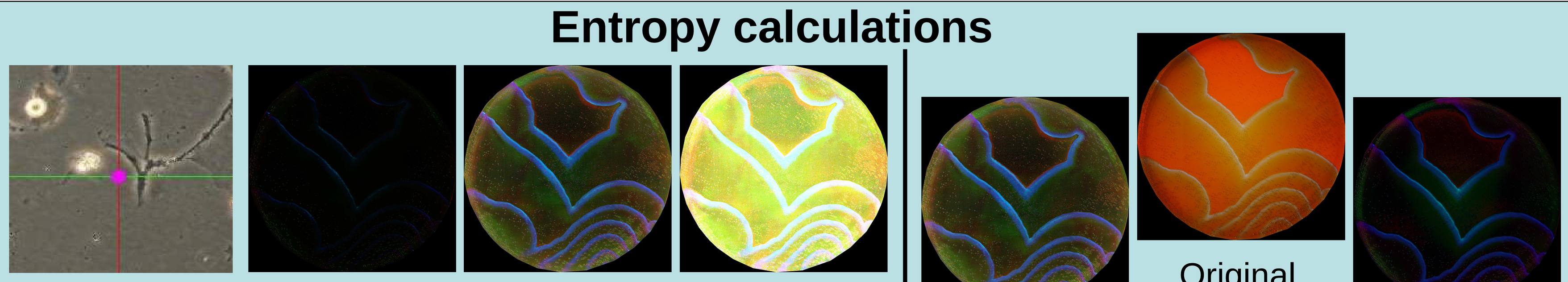
- ❑ Two computing methods – Whole image x Cross
  - ❑Whole image – Histogram is constructed from whole image
  - ❑Cross – Histogram is constructed from cross, more correlated with spatial and structural information
- ❑13 alpha parameter (ΔI_α) x 3 color channels = 39 dimension of state space

Point Information Gain Density

$$\Delta I_\alpha = \sum_{n=0}^{n=\max level} \Delta I_{x,y,\alpha}$$

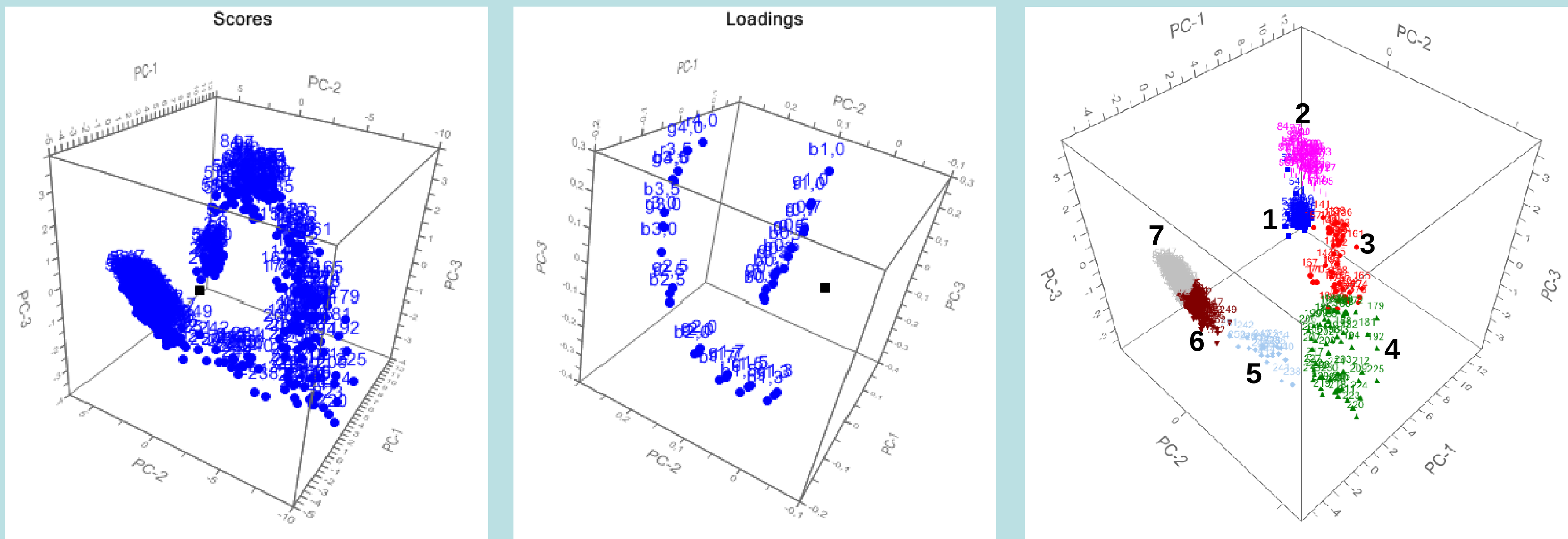
- ❑Clustering of trajectory – different algorithms
- ❑Hierarchical x K-Means
- ❑Number of clusters

Entropy calculations



Principle of cross computation | Cross α=0.3 | Cross α=1.0 | Cross α=3.5 | Cross α=1.0 | Original image | Whole image α=1.0

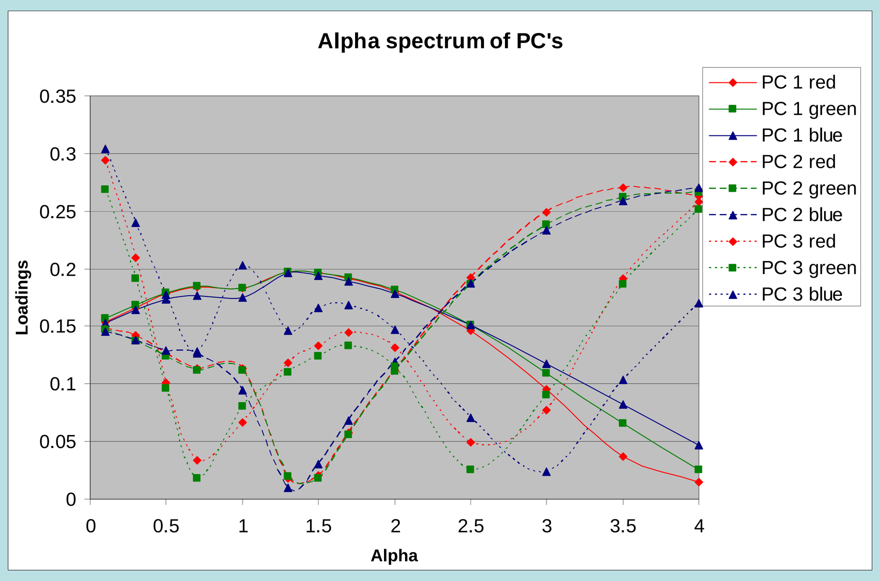
Principal component analysis - Cross

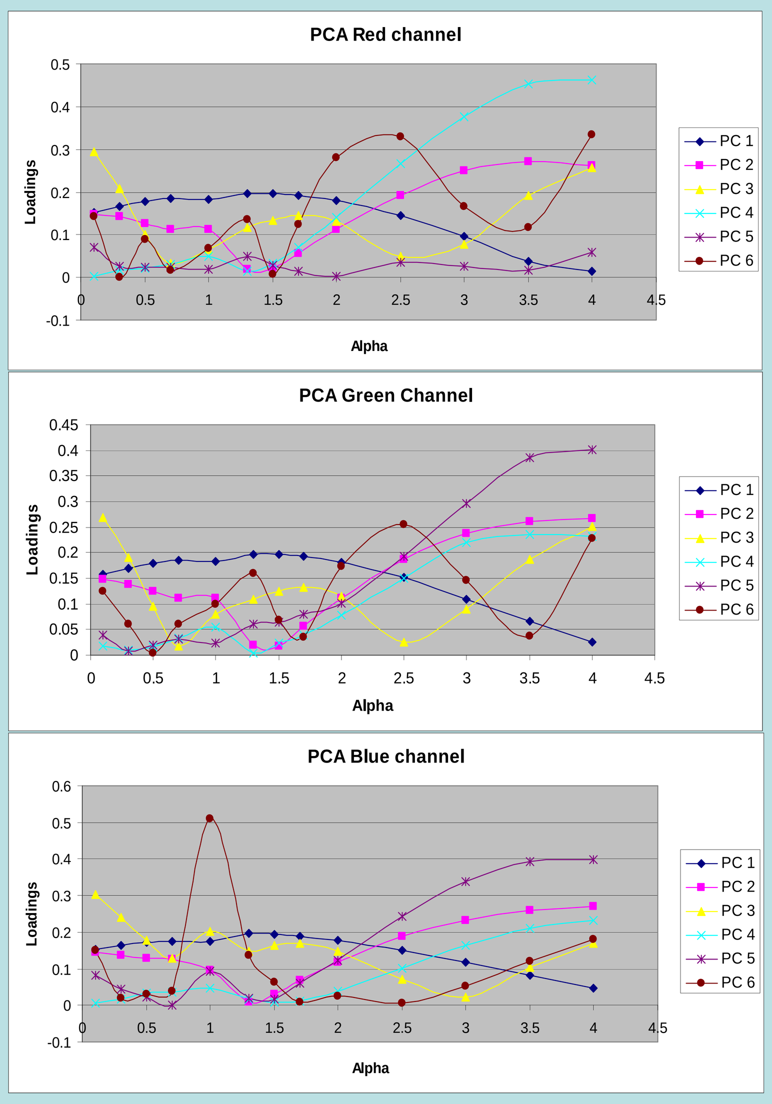


- ❑ Principal Component Analysis (PCA)
- ❑ Objective reduction of multidimensional space
- ❑ For cross – first 3 PCs – 97.25% of original space
- ❑ For whole image – first 5 PCs – 94.15%
- ❑ Spatially separated parts of state trajectory → easier clustering

PC-1	63.67%
PC-2	92.83%
PC-3	97.25%
PC-4	98.09%
PC-5	98.40%

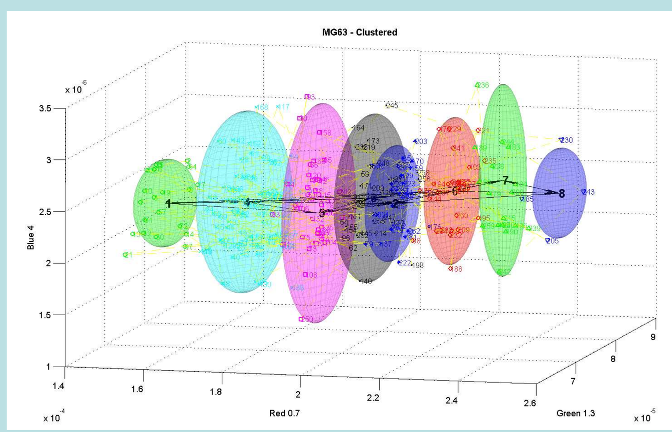
Alpha Spectra of Principal Components (PC)

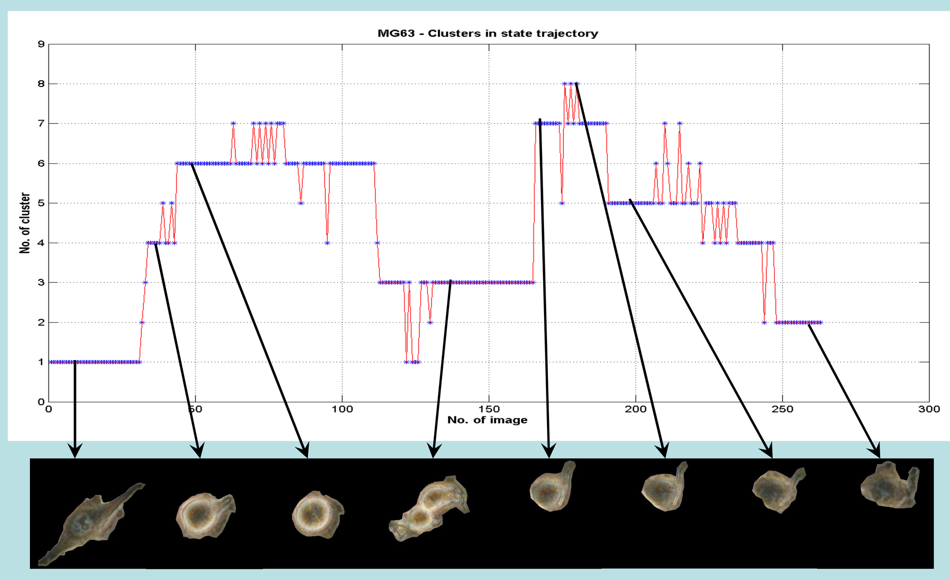




- ❑ Alpha spectra – which parts of image dominates PC
- ❑ Differences between PCs (upper graph)
- ❑ Differences between color channels (right graphs)

State trajectory of the MG63 cell





- ❑ Manually and subjectively constructed trajectory
  - X – axis Red channel with α=0.7
  - Y – axis Green channel with α=1.3
  - Z – axis Blue channel with α=4
- ❑ Number of clusters was only subjectively estimated – 8 clusters
- ❑ Manually extracted cell from image
- ❑ Time dependent changes of clusters
- ❑ Each cluster contains different images
- ❑ Need of objectively chosen alpha parameters (coordinates)
- ❑ Need of objective number of clusters

Discussion and Conclusions

- ❑ Algorithms were tested on BZ reaction model under different conditions
- ❑ The input for these algorithms was only image of BZ reaction without the background and Petri dish
- ❑ Constructing and clustering of state trajectory was successfully done
- ❑ Number of clusters for BZ reaction was objectively determinates by PCA
- ❑ Application of these algorithms for cells and intracellular object needs some preprocessing
- ❑ The preprocessing shall include mainly automatic identification of cell and automatic extraction of cell from image
- ❑ Construction of state trajectory of the cell is possible

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