Towards Automated Analysis of Belousov-Zhabotinsky Reactions

in a Petri Dish by Membrane Computing using Optic Flow

Benjamin Förster[1] Thomas Hinze[2]

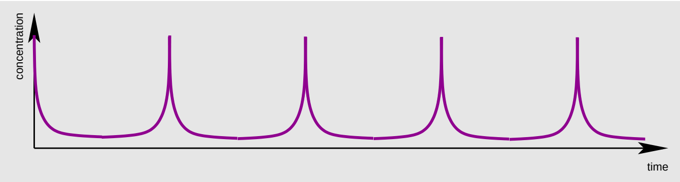
[1]Brandenburg University of Technology Cottbus–Senftenberg, Institute of Computer Science

[2]Friedrich Schiller University Jena, Department of Bioinformatics

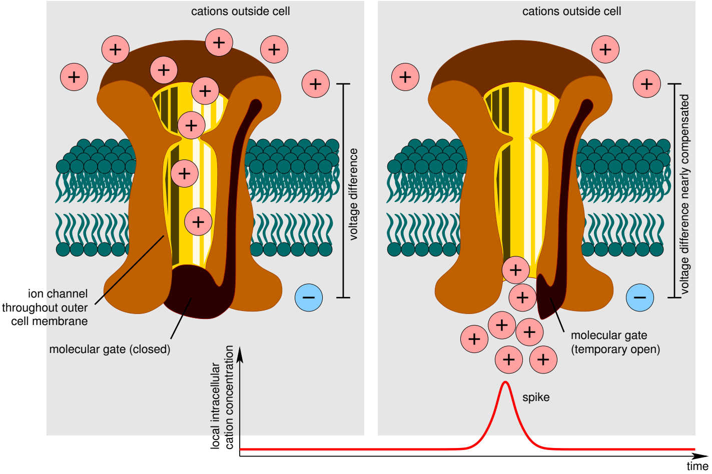
benjamin.foerster@b-tu.de thomas.hinze@uni-jena.de

Spiking Oscillations in Time and Space

- Widespread medium for *signal transduction* in biology
- Highly *energy-efficient*
- Oscillation course easy to generate
- Number and/or periodicity of spikes expresses information
- Utilisation of *frequency encoding* in biology
- Outstanding robustness against environmental perturbations and weakening of the signal when spreading out in space

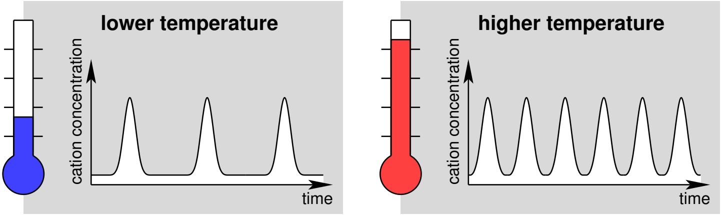


Example: Ion Channel-Based Temperature Reception



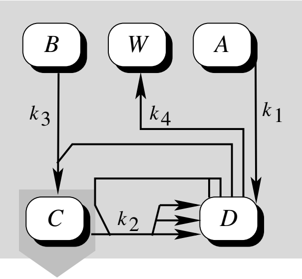
Transient Receptor Potential (TRP) channels highly conserved

Ion Channel Acting as Thermosensor



- With increasing temperature, diminished electrical forces to open molecular gate within TRP channel
- Increasing temperature results in higher frequency of spiking oscillation (warm sensor)
- *Frequency encoding* of temperature within physiological range but non-linear mapping between temperature and oscillation frequency

Reaction Scheme: Ion Channel as Thermosensor



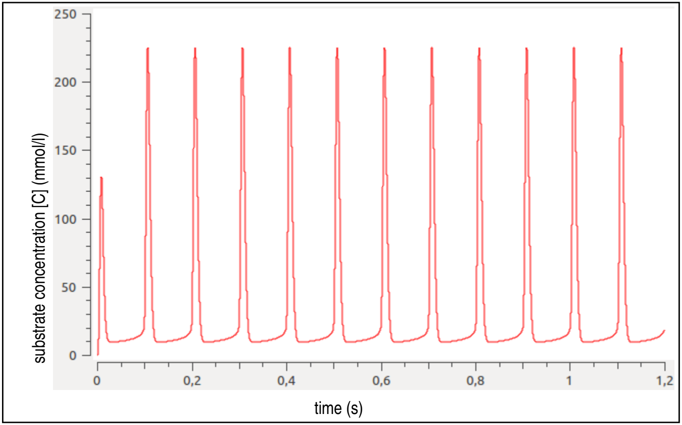
Species identifiers

A .... inositol triphosphate (IP3)
B .... calcium ions outside cell
C .... calcium ions inside cell (*output*)
D .... permeability of ion channel expressed by spatial protein structure
W .. waste (excess of open-gate *D* structure)

$$A \xrightarrow{k_1} D; \quad C + 2D \xrightarrow{k_2} 3D; \quad B + D \xrightarrow{k_3} C; \quad D \xrightarrow{k_4} W$$

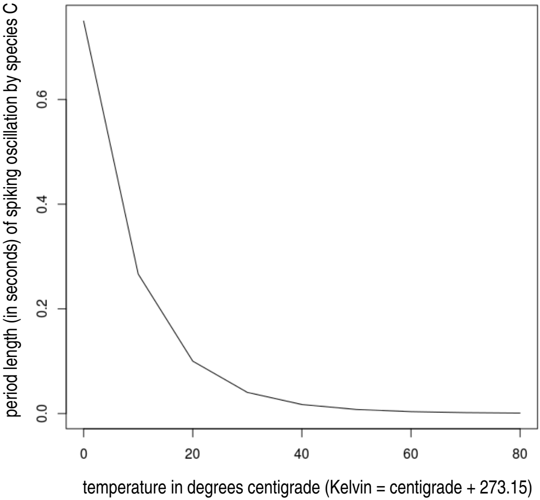
- *Suppliers* *A* (second messenger IP3) and *B* (Ca^{2+}) fuel the oscillator
- Self-amplifying effect attracts more and more *B* to *enter* the cell leading to fast increase of *C* (positive feedback induces spike)
- *Short-time self-amplification*, afterwards *collapsing* due to lack of *B*
- As soon as enough *B* accumulated, *next spike* generated
- Resembles operation principle of *Brusselator*

Spiking Behaviour of Thermosensor



- At 20°C (293.15K) spiking period length of 100ms
- Higher temperature shortens period length
- Thermosensor maps temperature into period length

Period Length subject to Environmental Temperature



Impetus of Spiking Oscillations in Biology

Found in numerous signal transduction schemes:

- *Ion channels* as sensors
- *Calcium oscillations* for intracellular signal propagation

Impetus of Spiking Oscillations in Biology

Found in numerous signal transduction schemes:

- *Ion channels* as sensors
- *Calcium oscillations* for intracellular signal propagation
- *Neurotransmitters* for information exchange across neurons

Impetus of Spiking Oscillations in Biology

Found in numerous signal transduction schemes:

- *Ion channels* as sensors
- *Calcium oscillations* for intracellular signal propagation
- *Neurotransmitters* for information exchange across neurons

$\Longrightarrow$ Biological systems with common principle of operation

Impetus of Spiking Oscillations in Biology

Found in numerous signal transduction schemes:

- *Ion channels* as sensors
- *Calcium oscillations* for intracellular signal propagation
- *Neurotransmitters* for information exchange across neurons

$\Longrightarrow$ Biological systems with common principle of operation

Find a model system *in vitro* to study behaviour in detail

Impetus of Spiking Oscillations in Biology

Found in numerous signal transduction schemes:

- *Ion channels* as sensors
- *Calcium oscillations* for intracellular signal propagation
- *Neurotransmitters* for information exchange across neurons

$\Longrightarrow$ Biological systems with common principle of operation

Find a model system *in vitro* to study behaviour in detail

- Separation from other systems for isolated consideration

Impetus of Spiking Oscillations in Biology

Found in numerous signal transduction schemes:

- *Ion channels* as sensors
- *Calcium oscillations* for intracellular signal propagation
- *Neurotransmitters* for information exchange across neurons

$\Longrightarrow$ Biological systems with common principle of operation

Find a model system *in vitro* to study behaviour in detail

- Separation from other systems for isolated consideration
- Capability of easy measurement and observation of signal courses in time and space

Impetus of Spiking Oscillations in Biology

Found in numerous signal transduction schemes:

- *Ion channels* as sensors
- *Calcium oscillations* for intracellular signal propagation
- *Neurotransmitters* for information exchange across neurons

$\Longrightarrow$ Biological systems with common principle of operation

Find a model system *in vitro* to study behaviour in detail

- Separation from other systems for isolated consideration
- Capability of easy measurement and observation of signal courses in time and space
- Opportunity for automated analysis of behaviour subject to controllable reaction parameters

Impetus of Spiking Oscillations in Biology

Found in numerous signal transduction schemes:

- *Ion channels* as sensors
- *Calcium oscillations* for intracellular signal propagation
- *Neurotransmitters* for information exchange across neurons

$\Longrightarrow$ Biological systems with common principle of operation

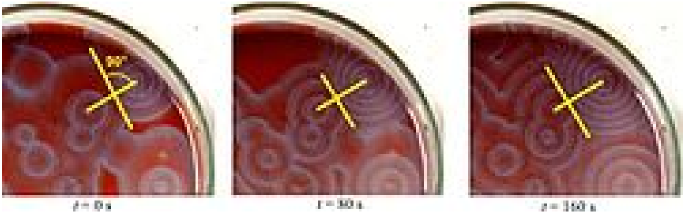
Find a model system *in vitro* to study behaviour in detail

- Separation from other systems for isolated consideration
- Capability of easy measurement and observation of signal courses in time and space
- Opportunity for automated analysis of behaviour subject to controllable reaction parameters

$\Longrightarrow$ *Belousov-Zhabotinsky* reaction scheme in a Petri dish

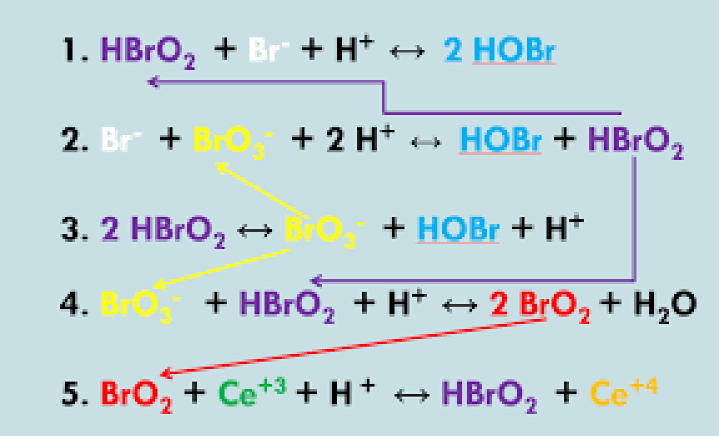
Belousov-Zhabotinsky Reaction Scheme in Petri Dish

- Dissipative auto-catalytic loop of two key processes
- Forward process generates molecular *bromine* (BrO_2, brown colour)
- Feedback process consumes bromine to release bromide ions (Br^-, grey or white colour)
- Injection of *ferroin, cerium or other indicator* acting as reductant to initiate oscillation
- Expanding *concentric rings* out of an *oscillatory spot*



www.wikipedia.org

Belousov-Zhabotinsky Reaction Network

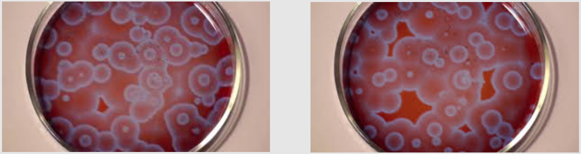


www.univr.it

Processes interact within positive feedback loop (Cerium injection).

Analysis of Belousov-Zhabotinsky Reactions

- *Videos* and *image sequences* document oscillatory behaviour
- Expanding concentric rings indicate run of the reactions
- Ratio of initial concentrations together with environmental factors like temperature determine oscillation frequency
- Simple *in-vitro* model for chemical *frequency encoding*
- Huge amount of video and image data available

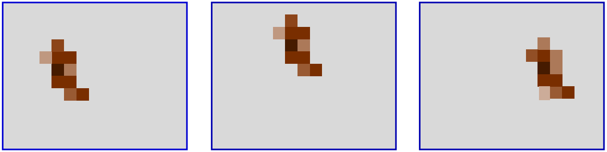


www.researchgate.net

$\Longrightarrow$ Aim: Automated analysis for identification and localisation of oscillatory spots and oscillation frequency in each spot

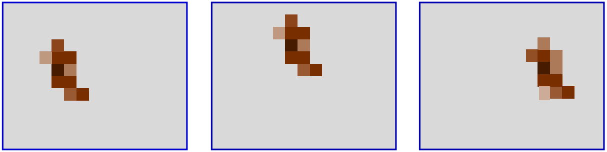
Application of Membrane Computing

- Groups of adjacent *pixels* in similar colour act as *particles*



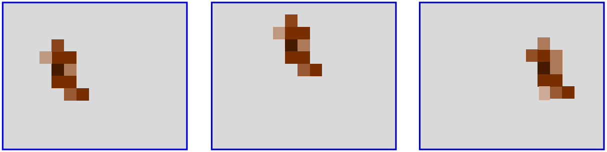
Application of Membrane Computing

- Groups of adjacent *pixels* in similar colour act as *particles*
- Movement of pixel groups (visual attributes) throughout a number of *subsequent images* within a sequence resembles passage of *membranes* by particles



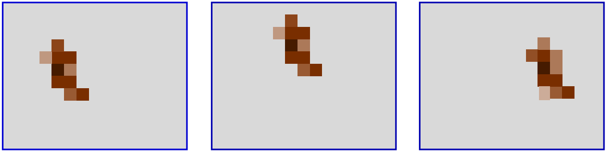
Application of Membrane Computing

- Groups of adjacent *pixels* in similar colour act as *particles*
- Movement of pixel groups (visual attributes) throughout a number of *subsequent images* within a sequence resembles passage of *membranes* by particles
- *Shape, intensity, or colour* of pixels in groups might slightly vary throughout a sequence of images $\longrightarrow$ *interactions or modifications* of particles when processed within or between membranes



Application of Membrane Computing

- Groups of adjacent *pixels* in similar colour act as *particles*
- Movement of pixel groups (visual attributes) throughout a number of *subsequent images* within a sequence resembles passage of *membranes* by particles
- *Shape, intensity, or colour* of pixels in groups might slightly vary throughout a sequence of images $\longrightarrow$ *interactions or modifications* of particles when processed within or between membranes



$\Longrightarrow$ Mathematical techniques for analysis of image sequences (Optic Flow) opens a new application of membrane computing.

Automated BZ Reaction Analysis

Goals

- Identify and count oscillatory spots
- Determine velocity of expanding concentric rings for each spot

[1]

Image Sequence Characteristics

What is missing?

- Image sequence of liquids $\Rightarrow$ constant illumination?
- No static background $\Rightarrow$ motion isolation?

What do we have?

- Small motion
- Neighbouring points move in almost the same direction
  - . . . but with slight intersection of the expanding concentric rings
- Stationary oscillatory spots
- Huge homogeneous areas

$\Longrightarrow$ Motion segmentation

Motion Detection and Segmentation

Goal

- Distinct motion areas around different oscillatory spots

Characteristics of a Method that would benefit us

- Works without foreground/background distinction
- Deals with changing illumination
- Can handle homogeneous, expanding areas
- Filters superposition of motion
- Robust to noise

$\Longrightarrow$ Looking for an egg-laying, milk-bearing woolly sow

Motion Detection and Segmentation

. . . with resulting vector fields

Method Overview

- Image difference
  - Needs homogeneous motion areas/rigid bodies
  - Extremely sensitive to noise
- Block matching
  - Divide image into macro blocks and estimate homogeneous motion for each block
  - Erroneous method
  - Can erase critical motion areas
- Optic Flow

Optic Flow

Characteristics

Advantages

- No fore- and background distinction needed
- Methods with partial robustness to noise exist
- Computes motion direction and velocity for each point, influenced by neighbouring points

Disadvantages

- Sensitive to variations in illumination
- Difficulties with homogeneous areas
- Sensitive to superposition of motions

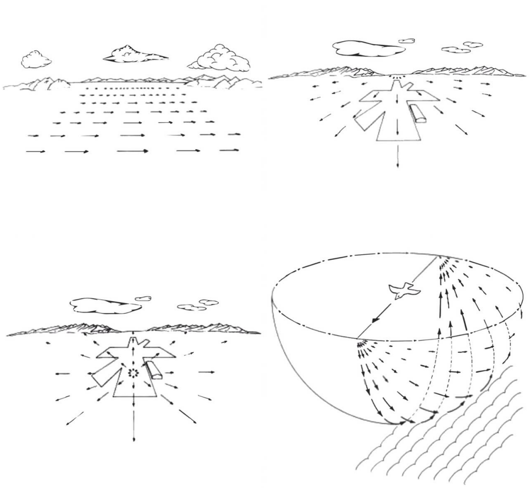
Optic Flow

Apparent motion of brightness patterns (Horn and Schunck [2]) . . .

- Usually computed on two dimensional grey-value (brightness intensities) sequences
- Results in a vector field

Optic Flow applications: (also holds for other motion segmentation methods)

- Autonomous driving, robot navigation and interaction with the environment (stereo vision)
- Image compression and reconstruction
- Tracking (e.g. optical computer mice)



James J. Gibson, The perception of the visual world [3].

Brightness Constancy Assumption

Constant Brightness Patterns

- Brightness of points in a pattern is expected to be constant

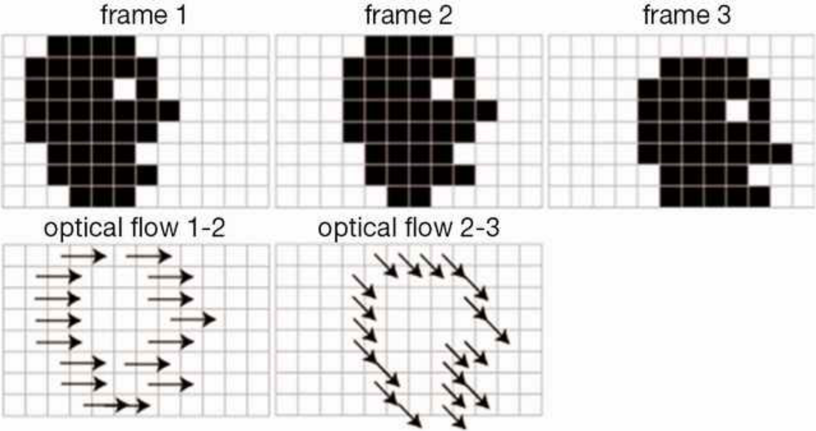
$$E(x(t), y(t), t) = C \text{ (constant)} \Rightarrow \frac{dE}{dt} = 0$$

- It follows a linear equation with two unknowns (u, v)

$$E_x \cdot u + E_y \cdot v + E_t = 0$$
$$u = \frac{dx}{dt},\ v = \frac{dy}{dt}$$

$\Longrightarrow$ Second constraint for motion vector determination needed

Brightness Constancy Assumption

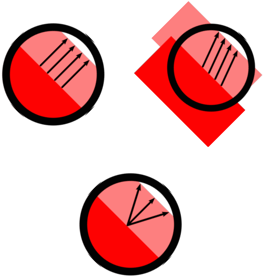


Example for a moving brightness pattern. [4]

Aperture Problem

Barberpole Illusion

- First observed and evaluated by Hans Wallach in 1935 [5]



Barberpole [6]

- Various concepts for the second constraint we will use the proposal by Horn and Schunck [2]

Second Constraint

Problem

- each point moves for itself ⇒ recovering motions will be impossible

Solution

- assume that neighbouring points undergoing similar motions as the point itself and the motion field varies smoothly everywhere

$$\nabla^2 u = 0 \text{ and } \nabla^2 v = 0$$

- penalise deviation from expected smooth variation

Euler-Lagrange Equation

Minimise the Error

$$\mathcal{E} = \int \int \alpha^2 \cdot \mathcal{E}_1^2 + \mathcal{E}_2^2 \, \mathrm{d}x \, \mathrm{d}y$$

- Minimising the sum of the first and second constraint and a weighting factor α^2
- Equation will be transformed into a linear equation system and solved with a fixed point iteration scheme

Optic Flow Results

- Optic Flow motion vector field of BZ reaction sequence

Next Steps

- apply a filter to the Optic Flow result
- determine sources of the vector field

Recovering the Motion

Expected Motion Field

- Same velocities around an oscillatory spot
- Motion directions vary only slightly
  - ⇒ Expanding concentric rings
- all motion leading away from a common central point

Filter Expectation

- Recover the motion that outweighs an area
- Robustness against outliers
- Determine and preserve sinks

Smoothing Filter

$$\vec{V}_{m,n} = (\vec{V}_{t,x}, \vec{V}_{t,y}) \text{ with } 2 \cdot m \cdot n \text{ elements}$$

$$V_{t,x}(p,q),\ V_{t,y}(p,q) \in \mathbb{R} \text{ with } p = 1,\ldots,n \text{ and } q = 1,\ldots,m$$

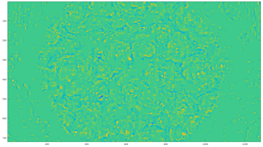
$$S = \begin{pmatrix} s_{1,1} & s_{2,1} & s_{3,1} \\ s_{1,2} & s_{2,2} & s_{3,2} \\ s_{1,3} & s_{2,3} & s_{3,3} \end{pmatrix} = \begin{pmatrix} \frac{1}{12} & \frac{1}{6} & \frac{1}{12} \\ \frac{1}{6} & 0 & \frac{1}{6} \\ \frac{1}{12} & \frac{1}{6} & \frac{1}{12} \end{pmatrix}$$

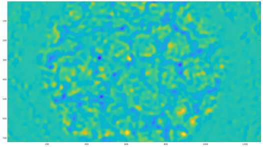
$$V_{t+1,x}(p,q) = \sum_i \sum_j s_{i+2,j+2} \cdot V_{t,x}(p+i, q+j) \quad \text{with} \quad i,j \in \{-1,0,1\}$$

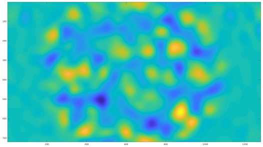
$$\text{for } V_{t+1,y}(p,q), \text{ respectively}$$

Resulting Flow Field

- $10\times$, $100\times$, $1000\times$ filter applications
- constraint to determine filter applications automatically



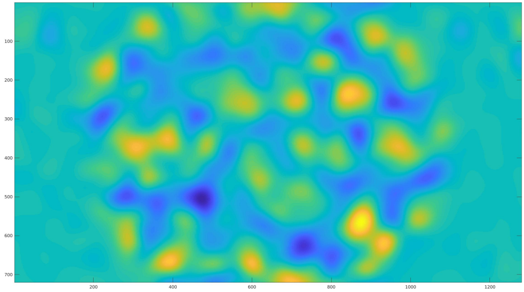




Vector Field Sources

Determine Sources and Sinks

- Divergence of a vector field results in a scalar field
- Each scalar represents how sourcish/sinkish a value is



Future Prospect

- Finalise the automatic evaluation to receive the number of osciallatory spots and velocity of the concentric rings around them
- Finalise an implementation with self-explaining user interface
- Test different Optic Flow approaches to reduce filter dependency

Thank you very much for your attention!

Bibliography

"Laboratory assistant."
"https://yubanet.com/wp-content/uploads/2017/09/09-20-2017-PAHO_082A0326.jpg", accessed 2018-08-31.

B. K. Horn and B. G. Schunck, "Determining optical flow," *Artificial intelligence*, vol. 17, no. 1-3, pp. 185–203, 1981.

J. J. Gibson, "The perception of the visual world.," 1950.

"Moving brightness pattern."
"http://tcr.amegroups.com/article/viewFile/3200/html/22837", accessed 2018-08-30.

H. Wallach, "Über visuell wahrgenommene bewegungsrichtung," *Psychologische Forschung*, vol. 20, no. 1, pp. 325–380, 1935.

"Barberpole image."
"https://openclipart.org/image/2400px/svg_to_png/175435/barber-pole.png", accessed 2018-08-30.