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Fast Artificial Intelligence for the Detection of Viral Particle Proliferation in Cellular Imaging Data

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Imaging Viral Proliferation

Immunocytochemistry and cell viability imaging by labeling techniques permit studying spatial and temporal dynamics of virus spreading on host cell surfaces \textit{in vitro}. 


Baby hamster kidney (BHK) cells were seeded on six well plates, grown as confluent monolayers and covered with a thin layer of agar. After piercing a small orifice in the agar, cell layers were infected by injecting 5 μl of virus VSVN1 inoculum with 1.6x10^7 infectious particles (a multiplicity of infection of 20). Cells were subsequently fixed, and immunofluorescence labeled with an antibody against a viral glycoprotein on and within the infected cells.

Images of the cells were then acquired by epifluorescence microscopy at low magnification using a high-resolution monochrome digital camera.

Images acquired at different moments in time \textbf{capture the temporal dynamics of infection spreading} in the cells.
RGB image coding highlights the critical spatial and temporal aspects of viral infection and proliferation on the host cell surface.

RGB coding facilitates the interpretation of experimentally labeled data by highlighting spatial and temporal changes signaling viral proliferation (progression) and, potentially, antiviral mechanisms (remission) permitting the infected host cell to recover after chemical treatment.

However, visual classification and interpretation of the image material is ultimately required.

This may involve guesswork when the spatiotemporal uncertainty in the model image contents is high.
Image Simulations

160 high resolution image simulations were computer generated on the basis of a first copy of the original low-resolution “ground truth” image from Haseltine et al.’s study.

Single-pixel computer control permitted implementing changes simulating further viral particle growth or recession in terms of the finest possible progressive change in time across images.

It took 30 minutes to reconstruct a model copy of the ground truth image and about 1 hour to generate the 160 image simulations of single pixel change.

In experimental host cell manipulation, visually undetectable signs of infection progression or remission, reflected by the smallest pixel changes in the model image, could occur within minutes after focal infection, or chemical treatment (therapy), of a cell *in vitro*. 
**Self-Organizing Map Model**

Neurons (models, $m_i$) topographically close in the map activate each other to learn from their common input $x$

SOM learning [2] is represented by

$$m(t+1) = m_i(t) + \alpha(t) h_{ci}(t)[x(t) - m_i(t)] \quad (1)$$

where $t$ is an integer, the discrete-time coordinate, $h_{ci}(t)$ the neighborhood function, which converges towards zero with time, $\alpha(t)$ the learning rate, which also converges towards zero with time and determines the amount of learning in each model neuron.

At the end of a winner-take-all learning process, each image input vector $x$ is associated with its best matching model $m_c$. The difference $(x - m_c)$ is a measure of how close a final SOM model representation is to the original input value. This difference is reflected by the *Quantization Error (QE)*

The average QE of all $x$ ($X$) in an input image is given by

$$QE = \frac{1}{N} \sum_{i=1}^{N} \|X_i - m_{c_i}\| \quad (2)$$

where $N$ is the number of input vectors $x$. 

Sensitivity to R-G-B Contrast Intensity And Polarity

The SOM (prototype represented to the left here) is described formally as a nonlinear, smoothly ordered map of contrast sensitive neurons where high-dimensional input data (here the pixel R-G-B) are mapped onto the elements of a regular, low-dimensional array. A real vector $x$ of $n$-dimensions is associated with each neuron in the SOM.

The final synaptic weights in the SOM are defined in terms of a three-dimensional output vector space representing each channel.

The magnitude and the direction of change in any of these channels from one image to another is reliably reflected by changes in the QE [3,4,5,6,7].

The difference between the input and its final representation in the SOM determines the quantization error (QE) in the output of the SOM analysis.
Experimental Analysis

- The training image for unsupervised SOM learning (training set) was the “ground truth” image shown previously in slide 4.

- The 160 images simulating viral particle proliferation/remission were then fed into a single SOM.

- After learning, the SOM-QE output was written into a data file.

- Output plots of SOM-QE where each output value is associated with the corresponding input image were generated.

- The data are then plotted in increasing/decreasing orders of SOM-QE magnitude as a function of the corresponding image variations (classification).

- The computation time of the SOM analysis was about 12 seconds per image.

- The precision of the SOM-QE classification was then compared with that of fully supervised (human-in-the-loop) classification of the same image data using Image-J’s RGB Mean.
Table 1. Linear regression analysis of SOM-QE and RGB Mean Classification Data

<table>
<thead>
<tr>
<th>Normality test (Shapiro-Wilk)</th>
<th>Linear regression ($R^2$)</th>
<th>Simulated Mechanism in Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Som-QE</td>
<td>RGB Mean</td>
<td>Som-QE</td>
</tr>
<tr>
<td>.42 passed</td>
<td>.05 failed</td>
<td>.99</td>
</tr>
<tr>
<td>.48 passed</td>
<td>.05 failed</td>
<td>.99</td>
</tr>
<tr>
<td>.53 passed</td>
<td>.05 failed</td>
<td>.99</td>
</tr>
<tr>
<td>.84 passed</td>
<td>.05 failed</td>
<td>.99</td>
</tr>
<tr>
<td>Cell Recovery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.42 passed</td>
<td>.05 failed</td>
<td>.99</td>
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<tr>
<td>.47 passed</td>
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<tr>
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<tr>
<td>.74 passed</td>
<td>.05 failed</td>
<td>.99</td>
</tr>
</tbody>
</table>
Conclusions

The results show that unsupervised, self-organizing map-based learning generates single-pixel precise, statistically reliable image model classification.

The SOM output metric (SOM-QE) used to that effect outperforms human computer-assisted image classification exploiting the RGB image mean.

A linear model predicts increase or decrease in the SOM-QE as a function of local changes in single image pixel contrast in millions of image pixels.

The hypothetical timescale of cell changes simulated by single-pixel changes in high-resolution images (of viral progression or cell recovery) in a single cell or a monolayer of cells is estimated in seconds/minutes.

With increasingly high resolution camera solutions, images of cells in vitro taken from a constant camera position every ten minutes could be fed directly into a computer for SOM-QE analysis.

Cell biologists could become able to detect significant changes within unprecedentedly short delays.
References


