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

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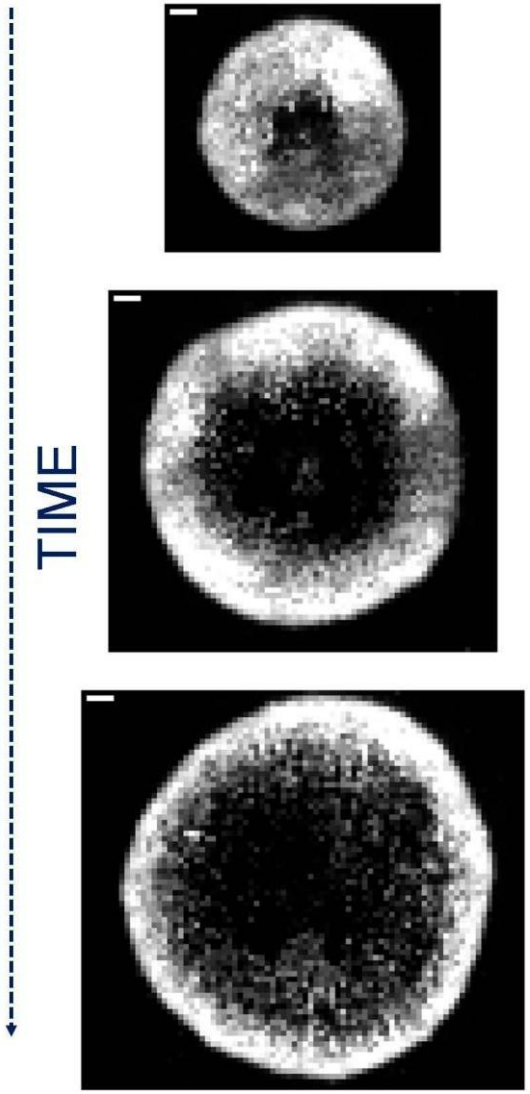
Imaging Viral Proliferation



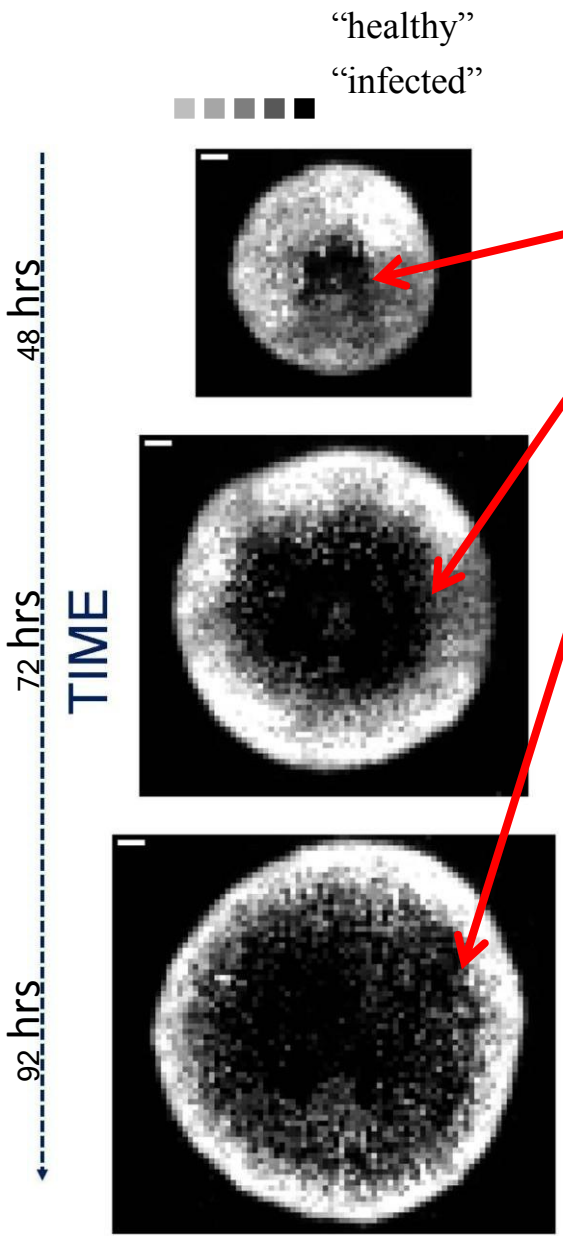
Immunocytochemistry and cell viability imaging by labeling techniques permit studying spatial and temporal dynamics of virusspreading on host cell surfaces *in vitro*.

Haseltine *et al.* [1] developed an imaging method that permits tracking the spread of focal viral infection using a combination of immunocytochemical labeling and step-by-step digital imaging.

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 1.6x107 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 epifluoresence microscopy at low magnification using a high-resolution monochrome digital camera.

Images acquired at different moments in time **capture the temporal dynamics of infection spreading** in the cells.



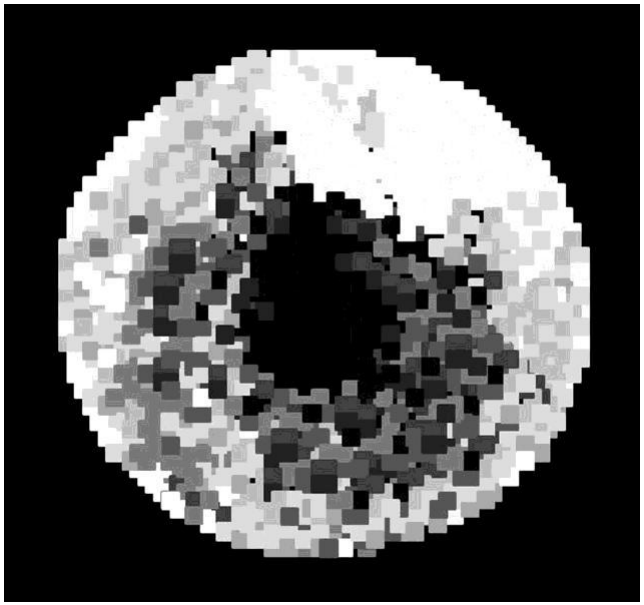
RGB image coding highlights the critical spatial and temporal aspects of viral infection and proliferation onthe host cell surface

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 ofthe image material is ultimately required

This may involve guesswork when the spatiotemporaluncertainty 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 lowresolution “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 changein time across images

It took 30 minutes to reconstruct a model copy of the ground truth image and about 1 hour to generate the 160image 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, $hci(t)$ the neighborhood function, whichconverges 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

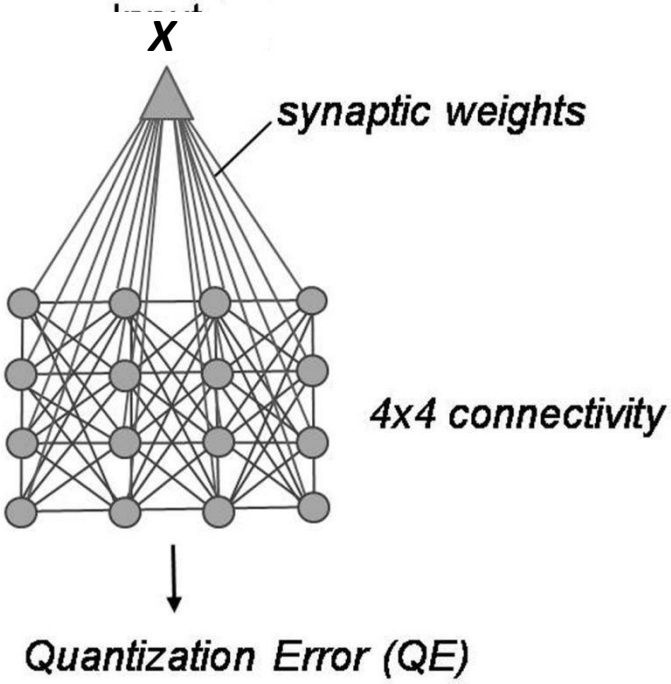
At the end of a winner-take-all learning process, each image input vector x *is* associatedwith its best matching model mc . The difference ($x - mc$) is a measure of how close a final SOM model representation is to the original input value. This difference is reflected by the *Quantization Error (QE)*

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

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

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 orderedmap 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 isassociated with each neuron in the SOM

The final synaptic weights in the SOM are defined interms 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 SOManalysis.

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 thenfed 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-QEmagnitude as a function of the corresponding image variations (classification).

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

- The precision of the SOM-QE classification was then compared with that of fully supervised (*human-in-the-loop*) classification of the same image datausing *Image-J's* RGB Mean.

Table 1. Linear regression analysis of SOM-QE and RGB Mean Classification Data

Normality test (Shapiro-Wilk)		*Linear regression (R^2)*		*Simulated Mechanism in Time*
SOM-QE	**RGB Mean**	**SOM-QE**	**RGB Mean**	
				Cell Infection
.42 *passed*	.05 *failed*	.99	.67	*black replacing white*
.48 *passed*	.05 *failed*	.99	.54	*dark gray replacing white*
.53 *passed*	.05 *failed*	.99	.32	*medium gray replacing white*
.84 *passed*	.05 *failed*	.99	0	*light gray replacing white*
				Cell Recovery
.42 *passed*	.05 *failed*	.99	.68	*white replacing black*
.47 *passed*	.05 *failed*	.99	.66	*light gray replacing black*
.60 *passed*	.05 *failed*	.99	.54	*medium gray replacing black*
.74 *passed*	.05 *failed*	.99	0	*dark gray replacing black*

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 models 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* froma constant camera position every ten minutes could be fed directly into a computerfor SOM-QE analysis

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

References

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