P28

Automated *In Silico* Discrimination of Murine Osteoclasts and their Precursor Cells

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Introduction

Osteoclast (OC) cultures are used to study the effect of drugs and xenobiotics on cell growth and function. The cultures contain OC and their immature precursors. Currently, human experts identify OC by counting the TRAP (tartrate-resistant alkaline phosphatase) positive cells. This is time-consuming and prevents the conduction of large-scale experiments. Neither can the amount of precursor cells nor the parameters like cell size in the culture be determined. The intra- and inter-variability between human experts is usually quite high. The major disadvantage of this method is that TRAP staining cannot be combined with other staining techniques to investigate protein-expression patterns in OC.

Applying image processing and machine-learning techniques in microscopic image analysis can dramatically improve the speed and reproducibility of cell identification. Automated analysis produces consistent quantitative measures of cell-associated parameters [1]. Using machine-learning techniques, tacit/intuitive knowledge which is applied but cannot be explained by human experts (see **KaryoQuest**) might be incorporated in algorithms. Finally, an automated analysis is more efficient than a human and can operate 24 hours and 7 days a week.

Aim

Development and validation of a versatile program that is capable of automated detection, quantification and characterization of multinucleated mouse osteoclasts and their precursor cells grown in culture based on virtual images derived from immunofluorescence (IF) microscopy.

Results

We created **two** *in silico* **approaches for OC-detection**:

"ClastoQuest" employs image-processing techniques and classifies the cells by cell-specific expression of proteins (F4/80) and their numbers of nuclei (Fig.1).

"KaryoQuest" uses machine learning to classify OC and precursors by their nuclei shape and texture only, without referring to other cell-specific markers (Fig.7, 8) .

Both approaches are currently subjected to extensive validation processes [3]. The ground truth data for the validations are generated by two human experts who independently marked OC (~150) in seven experiments (corresponding to 90 fields of view). These markups were automatically compared to the masks generated by ClastoQuest (Fig.4) and also served to characterize the identity of the analyzed nuclei by KaryoQuest (Fig.9).

Following their individual validation, KaryoQuest and ClastoQuest shall be combined to approximate highest possible performance in OC and precursor cell detection .

Summary

We have developed two approaches for automated detection of osteoclasts and their precursor cells in cultures which are based on different cellular features. Sofar, the approaches achieve accuracies of 86% and 75%, respectively, compared to human experts and shall be combinded to improve their performance.

Automated image analysis approaches

Approach 1 "ClastoQuest"

Details for mouse OC cell culture and IF-staining protocols can be found on the poster of Schepelmann et al (P65). **A typical input image is shown in Fig.1.** Some mature OC are indicated with green arrows. Nuclei are stained with DAPI (blue). The F4/80 macrophage marker that is lost upon differentiation of precursor cells to OC is shown in red. Alpha-tubulin and the calcitonin recepter (white) are stained to detect the area of all cells.

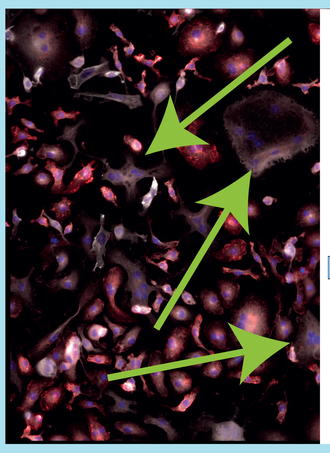


Figure 1

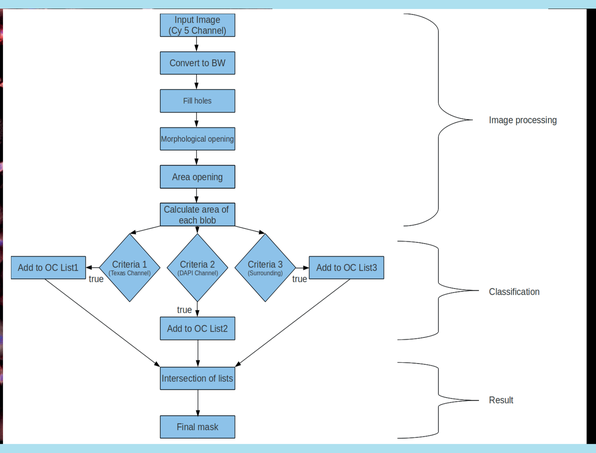


Figure 2

Figure 3

ClastoQuest consists of three main steps (Fig.2).

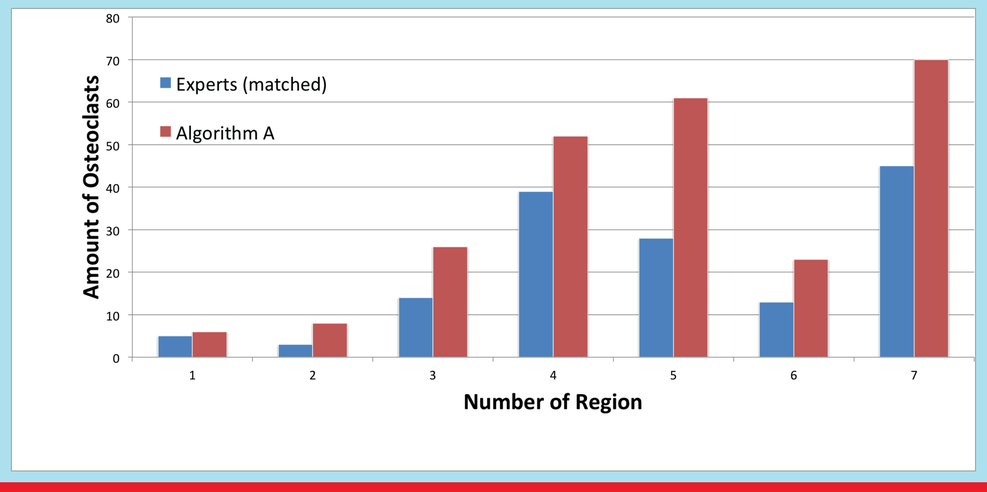
1/ Image Processing. Via a binary mask, morphological as well as area opening steps all cells are separated from background and small artefacts and disturbances are removed.

2/ Classification of cells (OC, OC precursor cells) by three criteria unique to OC (the algorithm criteria to detect OC correspond to the biological criteria of low expression of F4/80 and presence of at least 3 nuclei). Every cell is assigned with an unique identification number. An OC-mask corresponding to Fig.1 generated by ClastoQuest is shown in Fig. 3.

3/ Result: For each cell (OC and precursors) measurements such as area, or mean/median and standard deviation of fluorescence intensity (correlating to the protein expression under investigation) are computed.

Validation of ClastoQuest (Fig. 4) in 7 experiments. The blue bars show the amount of cells that were unequivocally classified as OC by two experts. The red bar is the amount of cells detected and classified as OC by ClastoQuest. **86% of OC identified by the human experts were also classified as OC by ClastoQuest,** but in most experiments, **ClastoQuest classified more OC than the human experts**. To decrease the number of false positives, ClastoQuest will be combined with KaryoQuest.

Figure 4



Approach 2 "KaryoQuest"

This cell classifier is based on the human observation that OC nuclei and non-OC nuclei appear differently. The human observers, however, were not able to describe the nature of the difference.

Two human experts marked up single nuclei in the DAPI channel (Fig.6) of IF-images (Fig.5) and these **perimeters were then analysed by a machine-learning system**. To fit these free-handed drawn perimeters to mathematically easier describable shapes, we **employed ellipses** (Fig. 7).

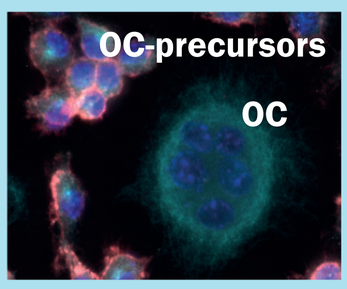


Figure 5

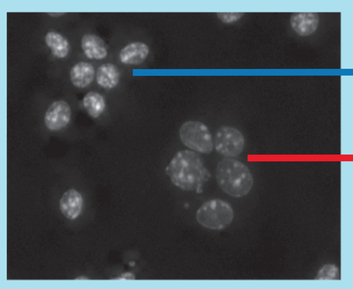


Figure 6

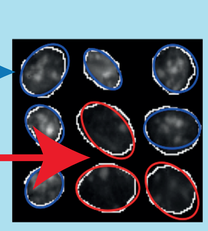
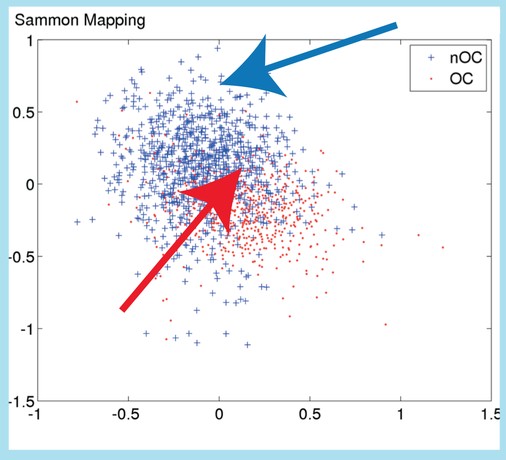
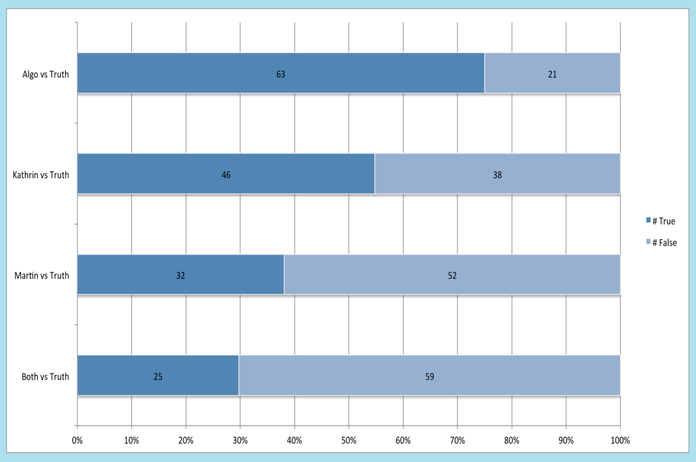


Figure 7

Various features were then computed from these ellipses such as mean of intensity, standard deviation of intensity, area, eccentricity, ... Though no single feature resulted in a clear separation of OC and precursor nuclei, a sammon-mapping [4] of results from all parameters - a procedure that maps this high dimensional space into a low dimensional space - showed that **nuclei of OC and precursors can be clustered upon combination of all available parameters** and thus be distinguished by KaryoQuest (Fig. 8)

Validation of KaryoQuest: Fig. 9 shows the performance of KaryoQuest and 2 human experts that detected 86 nuclei without any cellular context. Their classification of nuclei in OC or non-OC nuclei was validated versus an original ground truth generated by human experts seeing nuclei in their cellular context. Compared to this ground truth, the **algorithm achieved an accuracy of 75%** correctly classified nuclei whereas the best expert only classified 55% nuclei correctly.





Literature

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