Towards an automated evaluation system of osteoclasts in cultures

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TISSUE GNOSTICS
MEDICAL & BIOTECH SOLUTIONS

SEEWALD SOLUTIONS

Why use an automated evaluation system?

- Automated analysis produces *consistent* quantitative measures.
- Small differences not visible to the human eye but eventually linked to disease state, can be detected.
- Measures *each cell* rather than producing a score for the entire image.

Why use an automated evaluation system?(ctd.)

- Immunofluorescence microscopy yields *simultaneously* many informative measures of cells (e.g. protein expression).
  - Major advantage compared to the commonly used histochemical TRAP (tartrate-resistant acid phosphatase) staining.

- Less labor intensive → higher throughput

~300 minutes ➔ ~15 minutes

manually computer-based

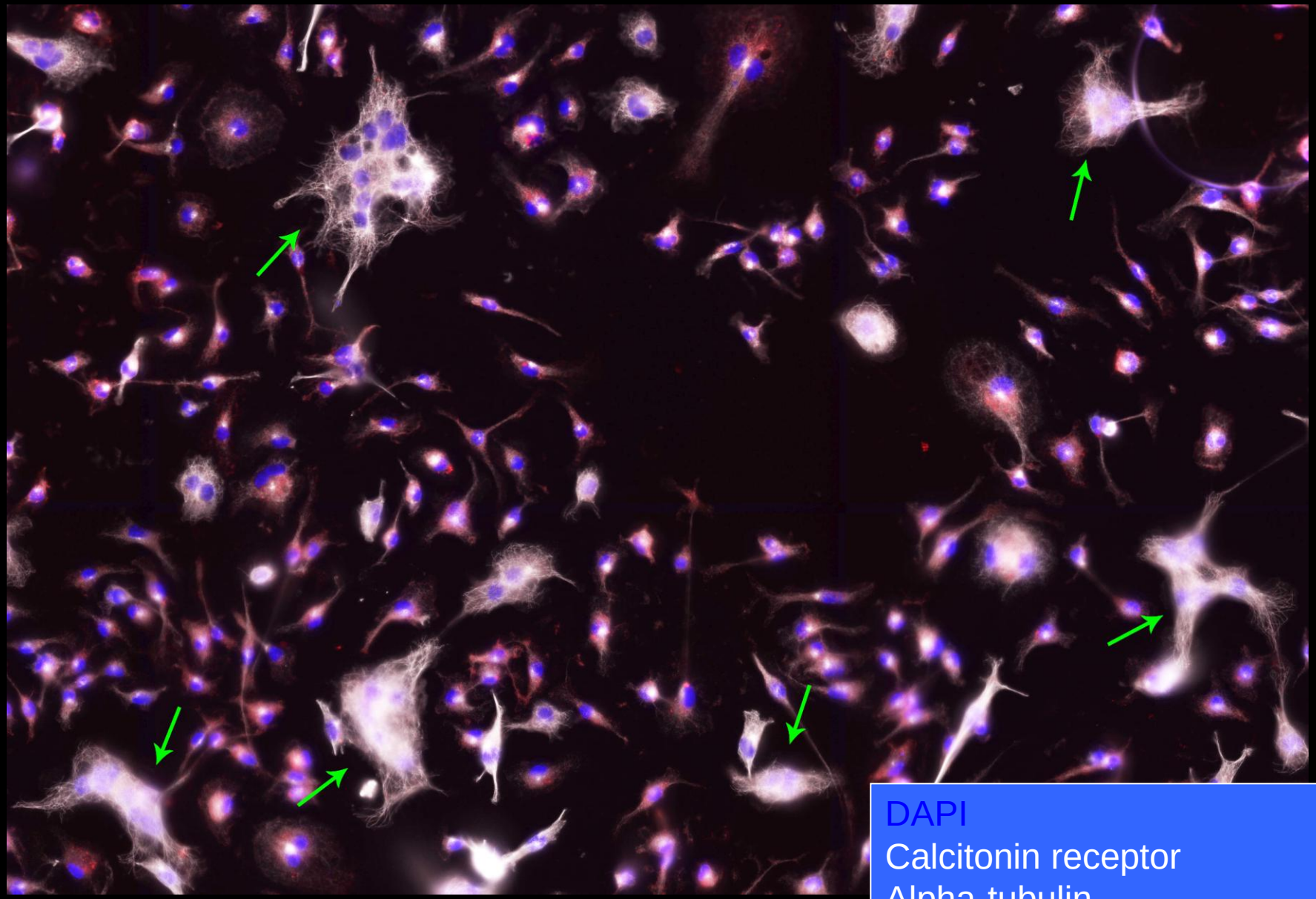
(Region size: 13920 x 10240 pixel, 20x)

Staining of osteoclasts and precursor cells

- Using an immunofluorescence staining protocol, osteoclasts cultures are stained as follows:

Precursor cells	
Nuclei	DAPI
Plasma membrane	Calcitonin receptor + Cy5
Cytoskeleton	Alpha-tubulin + Cy5
Osteoclast precursor cells	F4/80 Macrophage marker + TxR

Osteoclasts	
Nuclei	DAPI
Plasma membrane	Calcitonin receptor + Cy5
Cytoskeleton	Alpha-tubulin + Cy5


DAPI
Calcitonin receptor
Alpha-tubulin
F4/80 Macrophage marker

Algorithm to detect osteoclasts

- The specific staining of cells is used to classify cells in osteoclasts (F4/80-TxR – free) and non-osteoclasts (expression of F4/80-TxR).

- Cells classified as osteoclasts have to fulfill two osteoclast specific criteria.

Algorithm to detect osteoclasts(ctd.)

- Criterion 1 computes the ratio of the macrophage marker inside the cell versus the total area of the cell:

$$\frac{\text{amount(macrophage marker)}}{\text{area of cell}} < T_1$$

- If this ratio is lower than threshold T1 then the cell is added to the list α.

Algorithm to detect osteoclasts(ctd.)

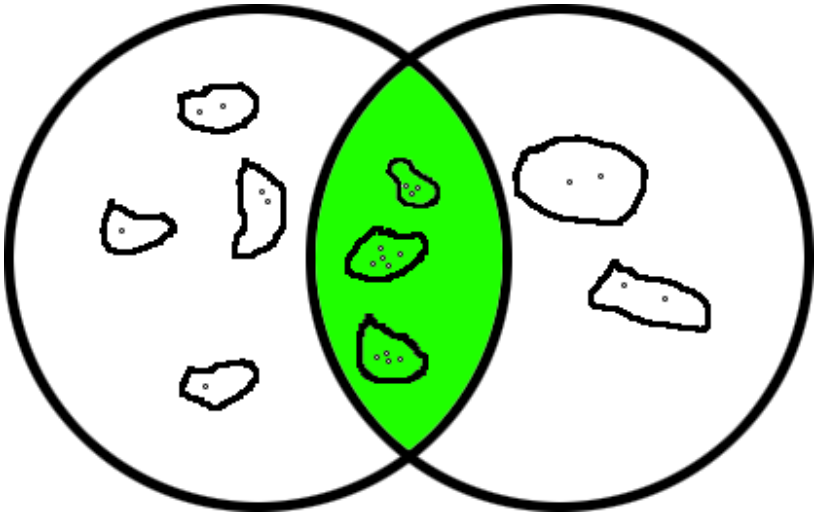
- Criterion 2 counts the amount of DAPI staining inside the cell:

$$area(DAPI) > T_2$$

- If this amount is higher than threshold T2 then this cell is added to the list β.

Algorithm to detect osteoclasts(ctd.)

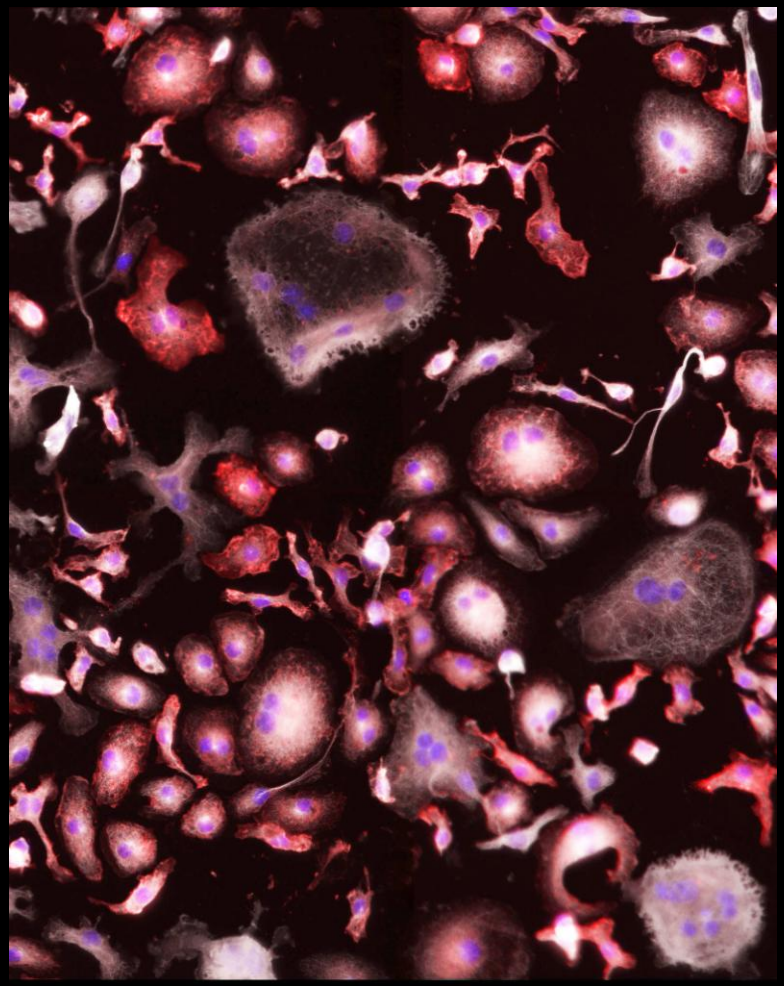
- Finally, those cells that are contained in both lists (intersection of list α and list β) are added to the final output mask.



List α

List β

Resulting output mask



What can be measured?

- Area of (each class of) cells per slide
- Amount of nuclei (per cell)
- Cell features:
  - Eccentricity
  - Perimeter, …
- Intensity-based features (e.g. protein expression)
  - Mean/Standard deviation
  - Entropy, …

For preliminary results: “A novel method for automated quantification of osteoclasts in culture – Advantages, workflow and application” by Martin Schepelmann