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DC VIS

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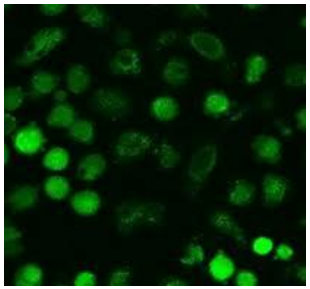
AUTOMATIC CELL COUNTER FOR CELL VIABILITY ESTIMATION

Overview

- Problem description
- Similar solutions
- CellCounter
- Empirical evaluation
- Future work

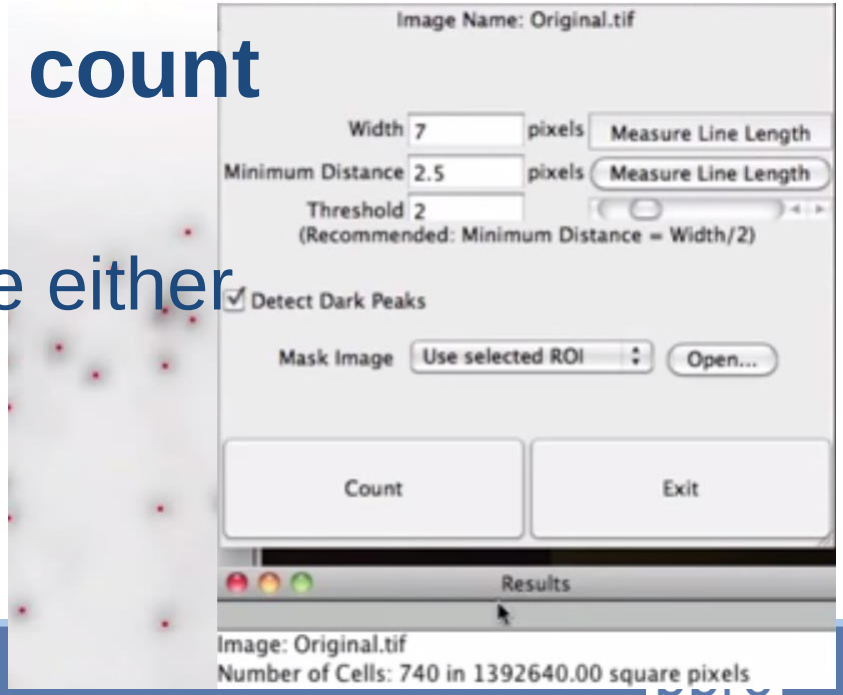
Problem description

- Counting of cells is still often used in analysis of:



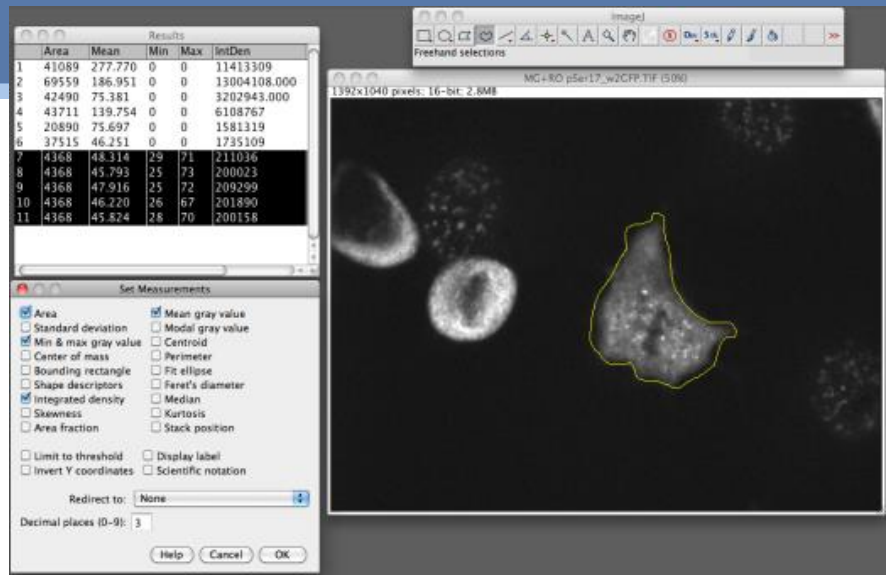
  - **drug** delivery, **transfection**, analysis of **mechanism**, detecting cell **viability**, **efficiency** of a specific drug, **delivery** or some other effect

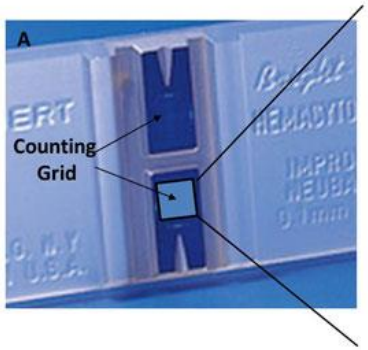
- many **researchers still count cells manually**
  - existing solutions require either
    - some *specific knowledge of computer vision* and/or
    - manual *fine tuning* of various parameters

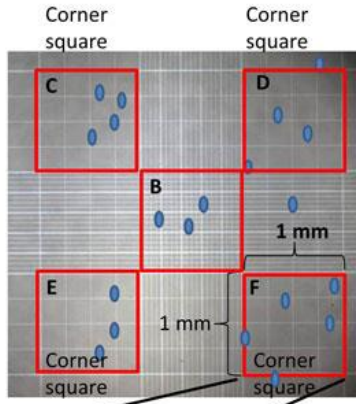


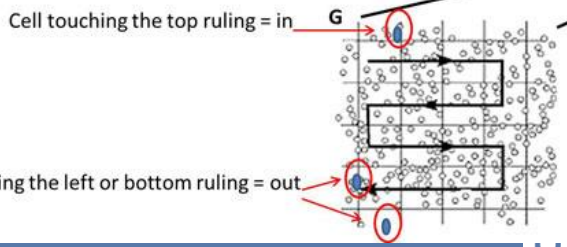
Existing solutions

- **Software solutions**
- Automated Cell Counting in ImageJ
- MetaMorph, BioQuant,
- Image-Pro, SynenTec
- CellProfiler, ImageTool
- …
- **Hardware solutions**
- LUNA automated cell counter
- Hemocytometer (Counting chamber)
- Flow cytometry
- …

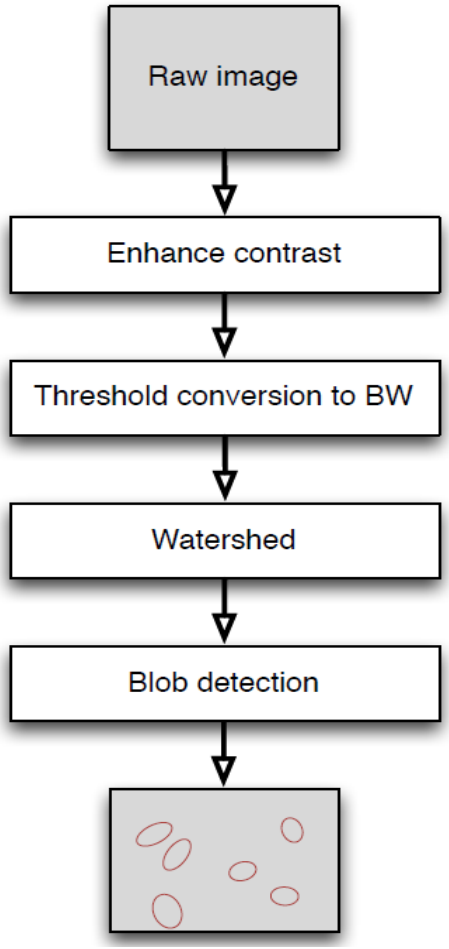




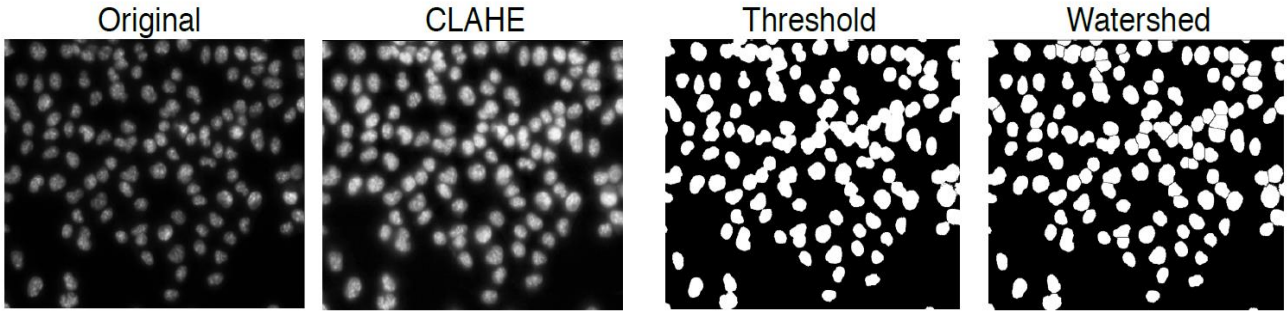




Proposed solution

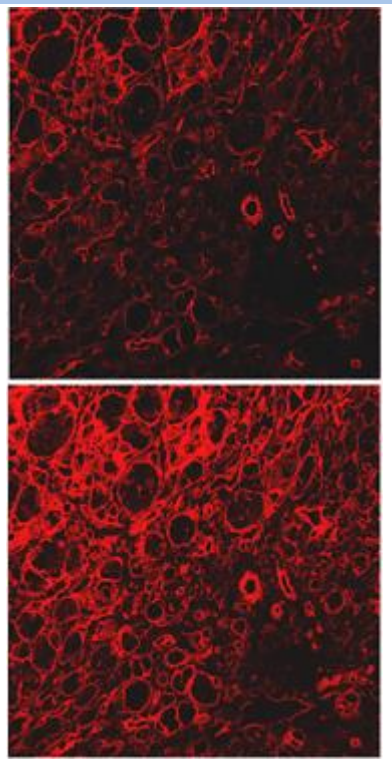


- A novel structured method for effective cell counting which **does not require any specific knowledge of image processing**.
- The method is specifically designed for **counting fluorescently stained cells**, that are used in many applications:
  - from measuring cell **viability** to determination of **transfection** efficiency



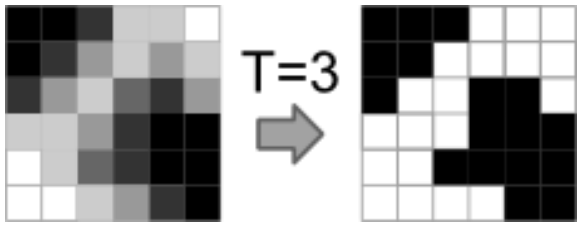
Finding the right contrast

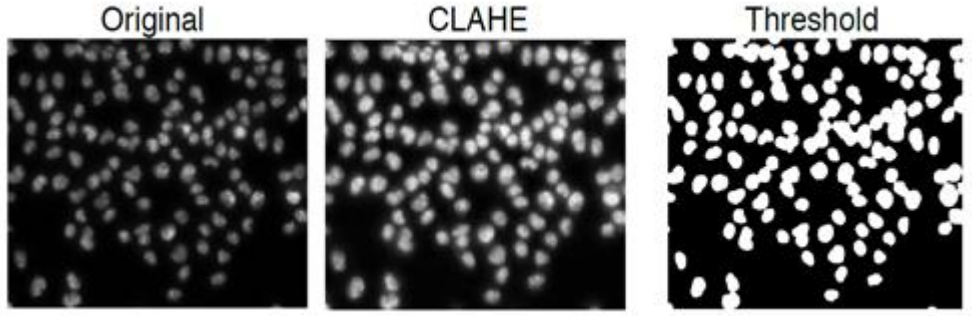
- **Contrast can be low** on microscopic images and **not distributed evenly** throughout the image due to **uneven illumination** or other optical problems
- image is improved using the **Contrast Limited Adaptive Histogram Equalization (CLAHE)** algorithm.
- It differs from ordinary histogram equalization in the respect that the adaptive method computes **several histograms**, each corresponding to a **distinct section of the image**
- It **redistributes** the brightness values of the image, In our solution the image is cut into **64 (8 × 8) disjoint sections** and histogram **equalization is applied on each part** independently
- **This method reveals more local information**



Threshold

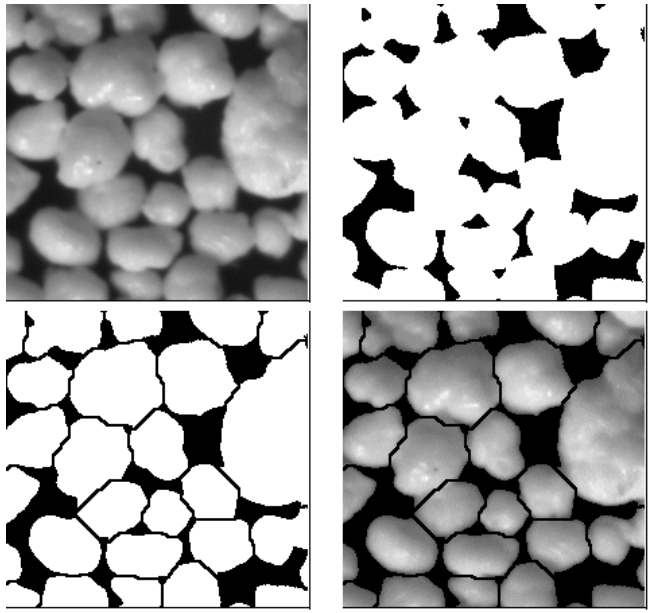
- Next step converts grey-scale image to black-white images where the object borders are defined using a **threshold algorithm**
- We use **Otsu** Threshold algorithm, since it gave slightly **better results** compared to other tested algorithms (Huang, Renyi entropy method, and other)
- It calculates the **optimum threshold** separating B&W classes so that their combined spread (**intra-class variance**) **is minimal**





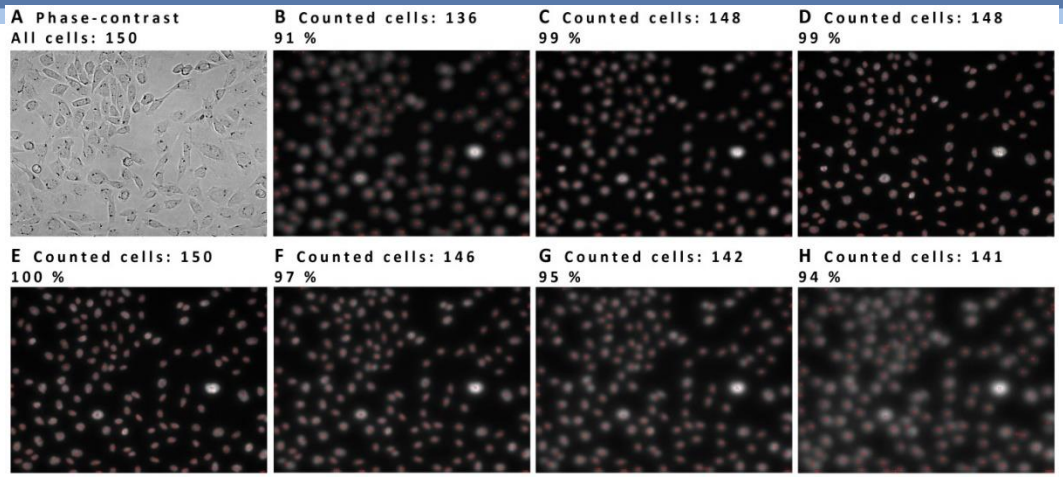
Spliting & detecting objects

- **Watershed algorithm** was applied. The image is **eroded** on the watershed lines, which usually represent **exactly the borders** between different cells.
  - the grey level of a pixel is interpreted as its altitude in the relief
- This enables the **detection** of
- **individual cells**, even **multi-nucleated** and **overlapping** cells
- connected regions are counted using **classical blob detection** algorithm, which counts the objects separated by black background

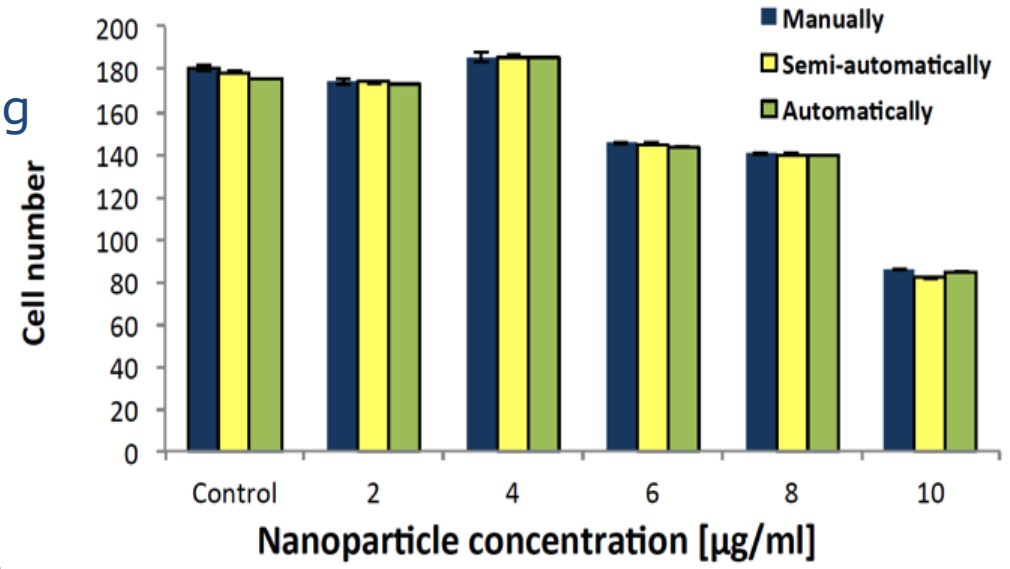


Empirical results

- A demonstration of the **robustness** of the program. The figures are images of Hoechst stained nuclei on the same visual field obtained on **different out of focus planes**

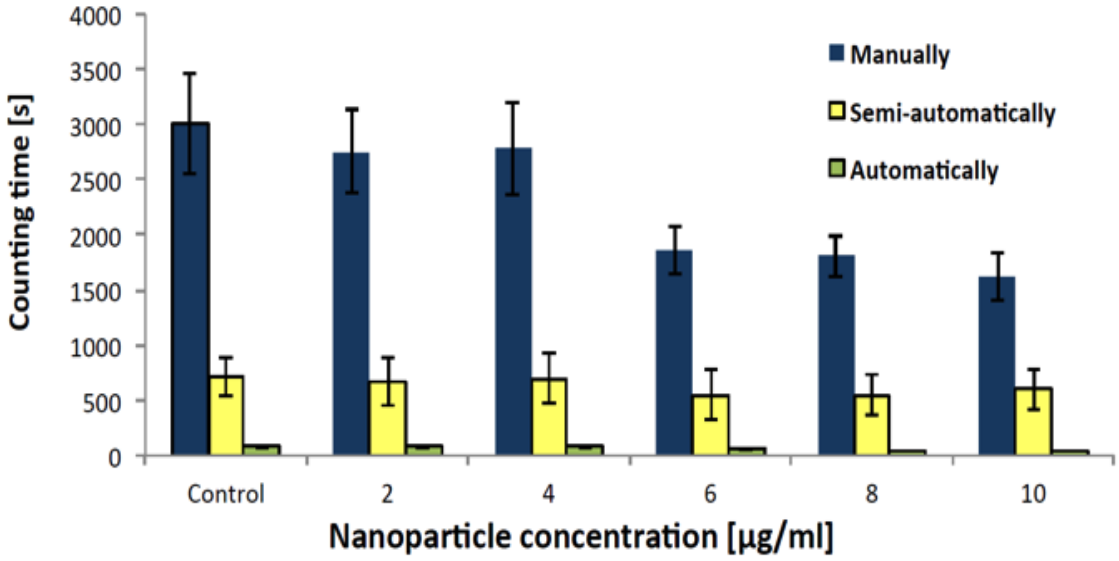


- Comparison of **counting results** obtained by counting fluorescently stained cell nuclei to determine the total number of CHO cells after exposure to increasing concentration of nanoparticles for 24 h



Assisting manual counting

- Although the **counting algorithm is robust**, as it is shown, the miscounts can still occur on more problematic images. To correct erroneously counted objects, a function was added to the user interface that enables the user to add or remove objects from the count with a mouse click. This is referred to as a **semi-automatic counting**



Conclusions

- In this paper, we present an **alternative solution for automatic and semi-automatic counting of cells** on **fluorescent** microscopic images.

- As we showed in this paper, the program enables **consistent**, **robust**, **fast** and **adequately accurate** determination of fluorescent cells and can therefore be **applied to a range of different applications** in different fields of life sciences where fluorescent labeling is used for quantification of different phenomena