Automated analysis of morphology attributes of PSCs in adherent and suspension culture

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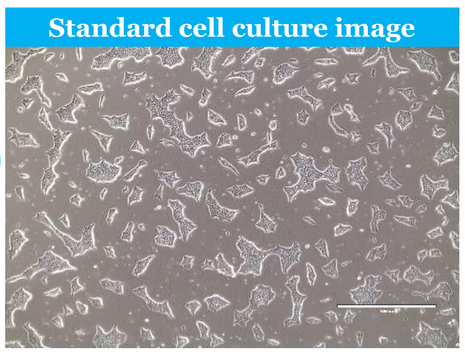
Introduction: Quality assessments based on operator judgements represent a development challenge for the industrial manufacture of cell therapy products derived from pluripotent stem cells (PSCs). The Cell and Gene therapy Catapult have adapted image analysis software to develop an automated, *in silico*, method that can rapidly and accurately measure both the confluence of 2D monolayer cultures and the size of 3D aggregates of pluripotent stem cells from the analysis of standard phase-contrast microscopy images. Our approach aims to reduce the process variability due to multiple operator judgments, to reduce labour requirements and process cost, and to increase the consistency of PSC-derived cell therapy products.

Adherent (2D)

Method: **(i)** Entropy filtering, **(ii)** Otsu Thresholding, **(iii)** Colony border identification, **(iv)** Halo/Edge artefact correction

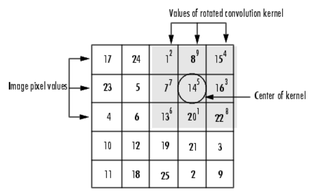
(i) Standard cell culture image



Phase contrast image of CGT-RCiB10 iPSC line (E8/VTN/Accutase). Scale bar: 1mm (x4 magnification).

High entropy

Entropy = 0

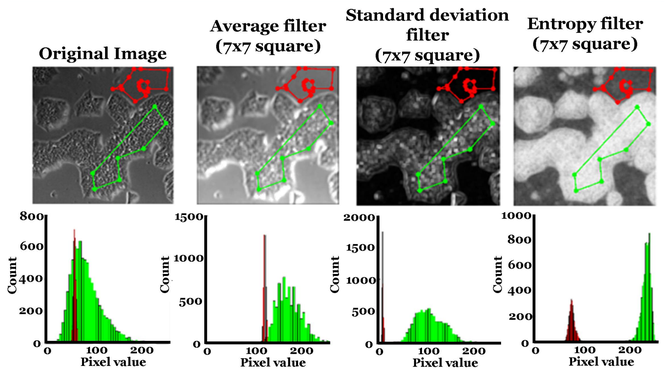


$$p_i = \frac{\text{number of pixels with intensity value i}}{\text{total number of pixels}}$$

$$Entropy = -\sum_i p_i \cdot \ln(p_i)$$

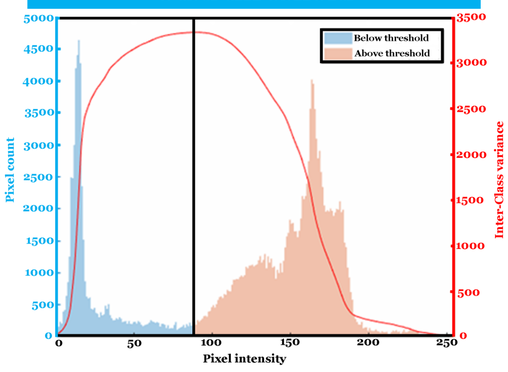
The borders of the cell colonies are identified by assessing the entropy for each pixel. Images with a large amount of complexity have pixels with a high entropy (colonies) , whereas those with little complexity have a low entropy value (background)[1]. This allows a border to be identified between areas of high and low complexity; the border between colony and background.

Image filtering



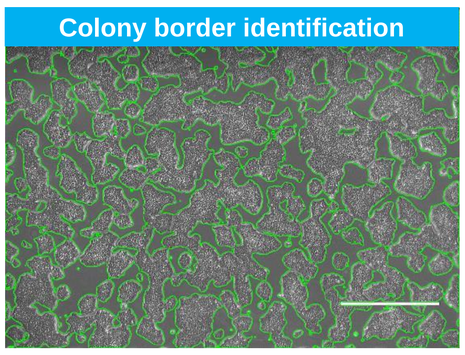
The cell colonies (green) can be segregated from the background (red) by applying the entropy filter. Measuring the pixel intensity, average pixel local pixel intensity, or standard deviation of local pixel intensity, insufficiently separates the colonies from the background. Entropy filtration gives two discreet populations for further analysis.

(ii) Otsu threshold



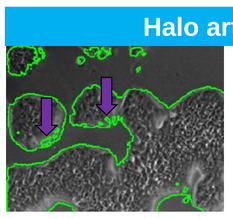
Otsu thresholding defines the point at which each population is defined. Above the threshold and the pixel is considered to be part of the cell colony. Below the threshold is considered to be background[2].

(iii) Colony border identification

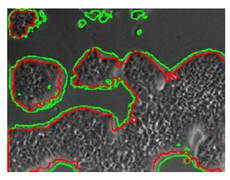


The borders of the colonies can be defined using the entropy filtration (green) to discriminate them from the background. Scale bar: 1mm.

(iv) Halo artefact correction



Poorly defined border artefacts (purple arrows) are present in areas where phase contract microscopy produces a 'halo effect' surrounding the colonies. These require correction.



Correction of the artefacts gives a more accurate representation of the colony border (red line). This increases accuracy the cell confluency estimation.

Analysis

Analysis

Analysis

Analysis

Colony confluency

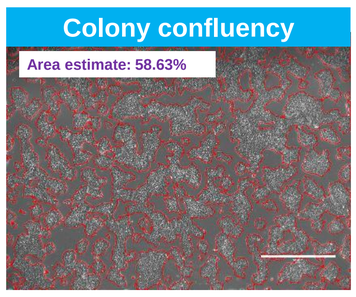
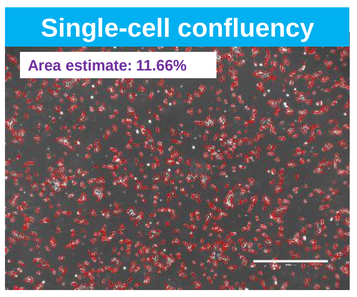


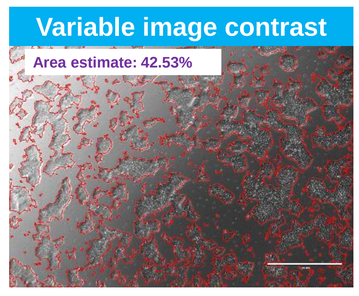
Image analysis software quickly provides an automated cell quantification expressed as a percentage of the overall image area. Scale bar: 1mm.

Single-cell confluency



The program demonstrates the capacity to measure both cell colonies and single cells (above). This is important given the increased implementation of single cell passaging methods in PSC manufacturing, which help to improve process consistency. Scale bar: 1mm.

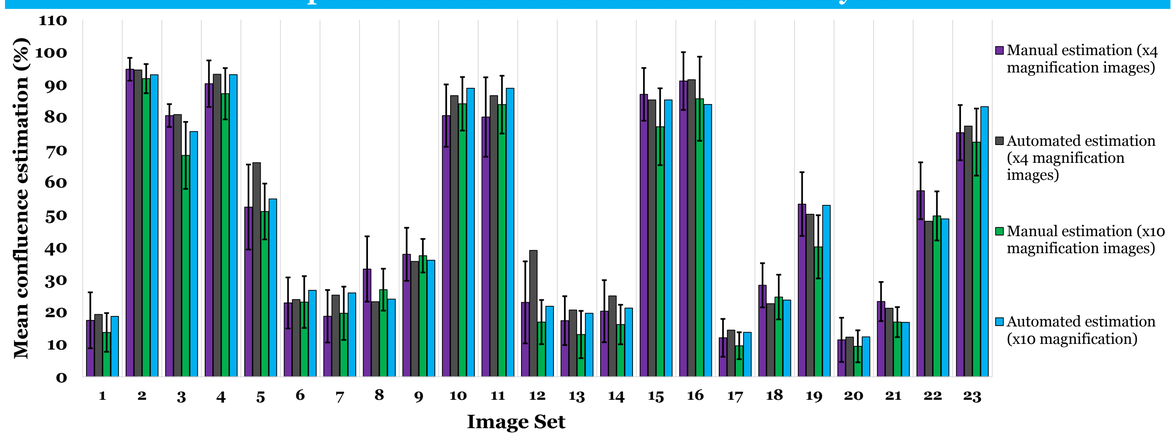
Variable image contrast



The analysis algorithm is able to quantify confluency in images with uneven contrast. Scale bar: 1mm.

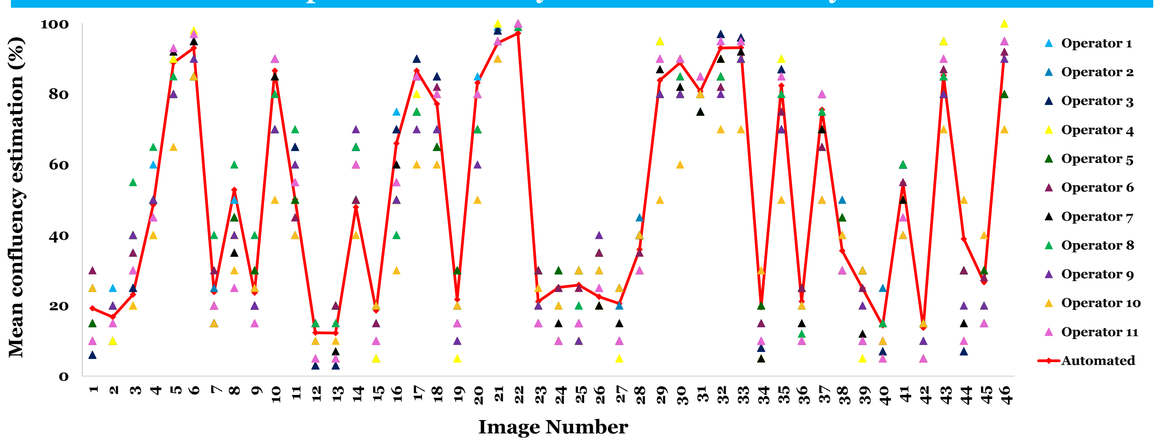
Manual vs. Automated Analysis: Confluency measurements from images at high/low magnification

Operator assessment and automated analysis



Mean values of confluency measured by automated analysis are equivalent to those estimated by experienced operators. The assessment can be made using images at either x4 or x10 magnification due to consistency observed across images taken at different amplifications. Error bars show the standard deviation (n=11).

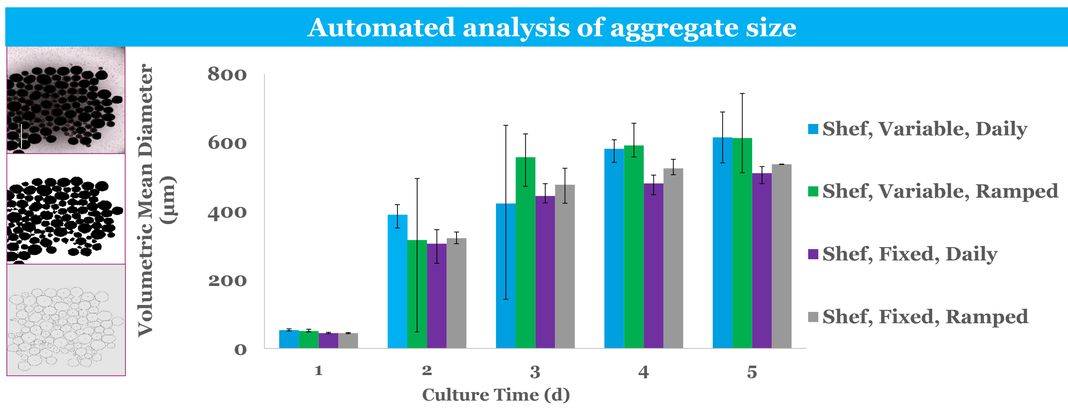
Operator variability and automated analysis



Inter-operator variabilities up to 35% are observed which highlights the requirement to automate and standardise assessment of morphology quality attributes and increases process consistency.

Aggregate (3D)

Automated analysis of aggregate size



ImageJ macro script was designed in-house to produce a mask surrounding each PSC aggregate. Particle analysis was then used to quantify the cross-sectional area and calculate the volumetric mean size of aggregate within the image (assuming each aggregate is spherical). Aggregate size was observed to increase over 5 days of culture Shef6.1 ESCs at different medium exchange regimes (Variable/Fixed, Daily/Ramped) in the AMBR15® microbioreactor. Error bars shows standard deviation (n=3).

Remarks

- **Image analysis algorithm for the reliable and automated quantification of cell confluency in 2D culture**
- **Automated analysis of 3D cell aggregate sizes**
- **Can be easily adopted for process control in real time**
- **Potential for greater consistency and control of PSC manufacturing processes.**

References: [1] Gonzalez, R.C., R.E. Woods, S.L. Eddins, *Digital Image Processing Using MATLAB*, New Jersey, Prentice Hall, 2003, Chapter 11; [2] Lucas(CA) - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=67144384

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