Electronic Supplementary Material (ESI) for Biomaterials Science.


**Supporting Information**

# Thin fluorinated polymer film microcavity arrays for 3D cell culture and label-free automated feature extraction

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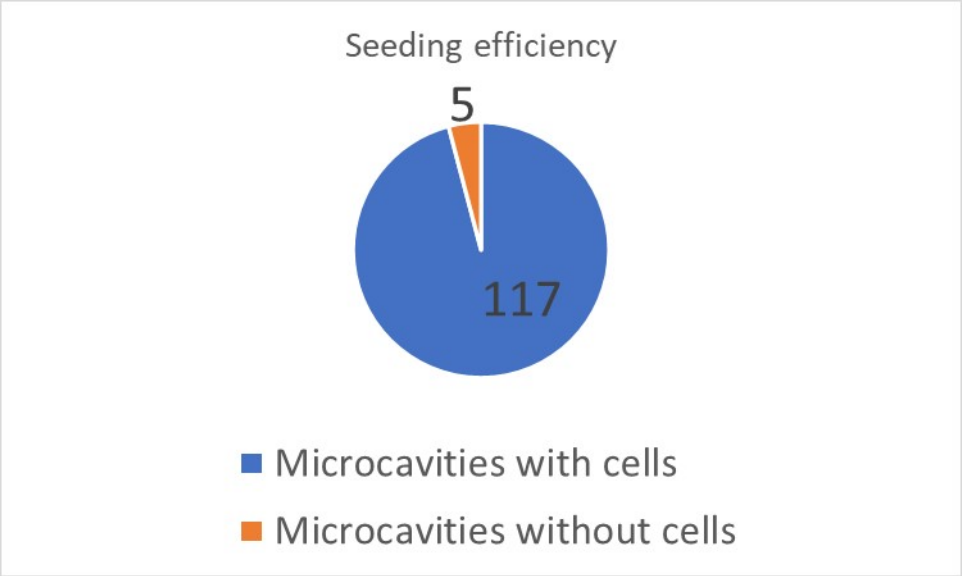


**Figure S1** Seeding efficiency in the microcavities was 95.9 percent when calculated across 122 microcavities spread across two different experiments. E14TG2A mESCs were seeded on the microwells as per the methods described in the experimental section. The microcavities were imaged using brightfield mode (Nikon eclipse Ti-S microscope) and the results were manually counted using FIJI.

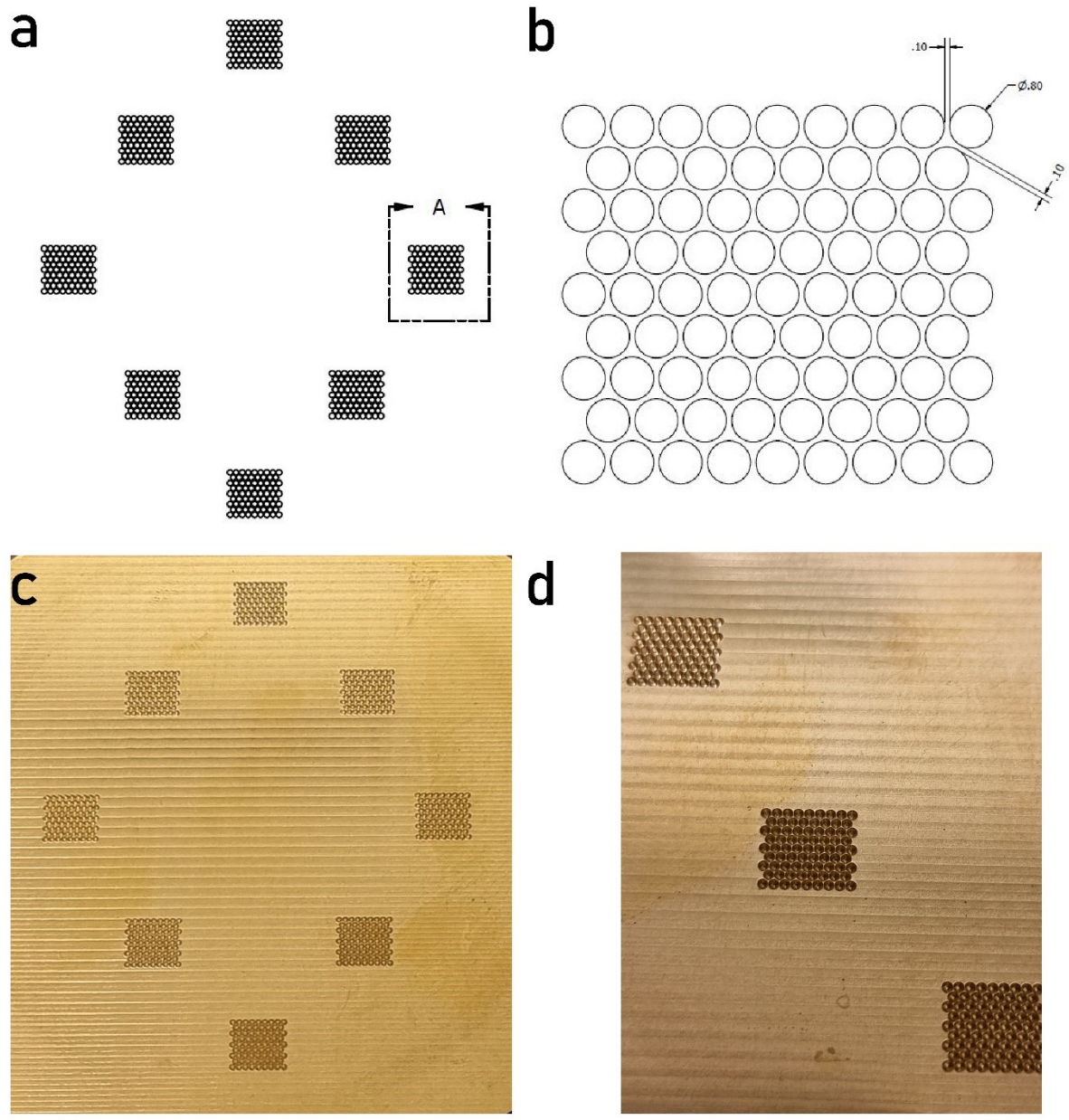


**Figure S2** Mold used for microthermforming. a,b) Design and more detailed image of a single array (inset box A) showing the dimensions (units in mm). c,d) Photos of the resulting mold and a close-up view of three microcavity arrays.

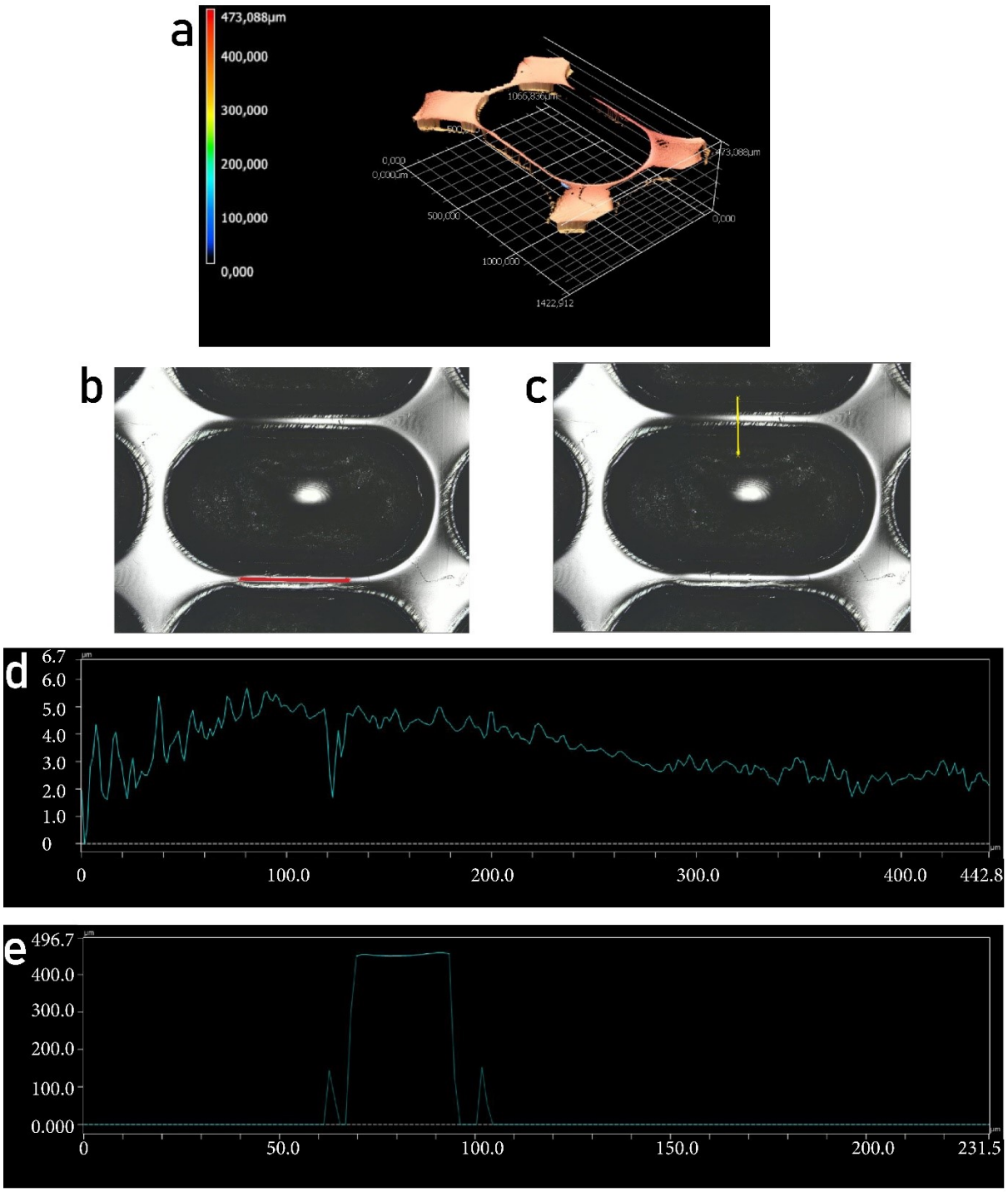


**Figure S3** Elongated FEP microcavities and height profile. a) 3D representation of the microcavity showed in Figure 2d. b) More detailed surface profiles for the same microcavity across the red line are shown in (d). c) Similarly, more detailed surface profiles for the same microcavity across the yellow line shown in e). All units are in µm.

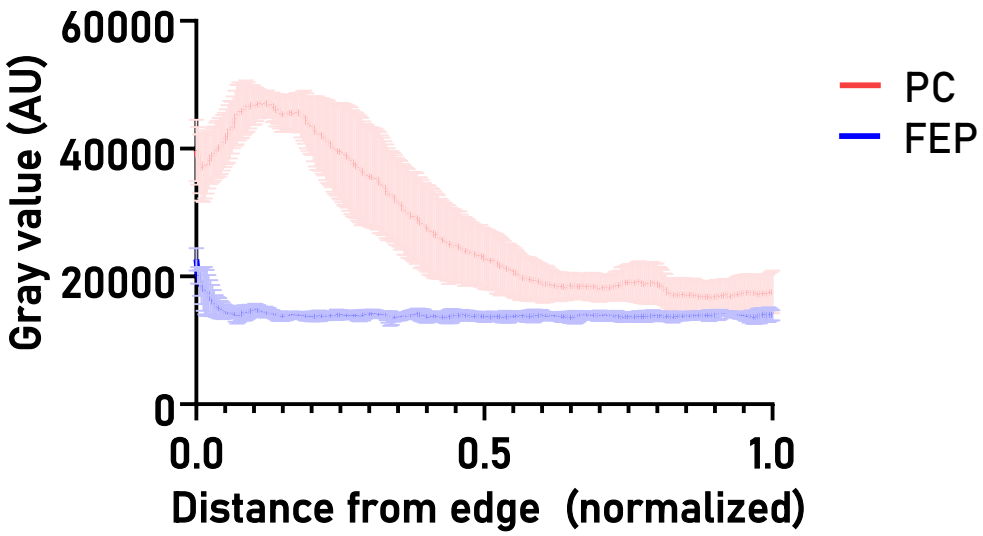


**Figure S4** Quantification of gray values across randomly selected FEP and PC cavities (N = 3) showing the standard deviation.

**Figure S5** Photo of FEP microcavities in cell culture media after seeding of H2B-RFP mESCs. As observed in the photo, the macroscopic well is illuminated using a light in the background.

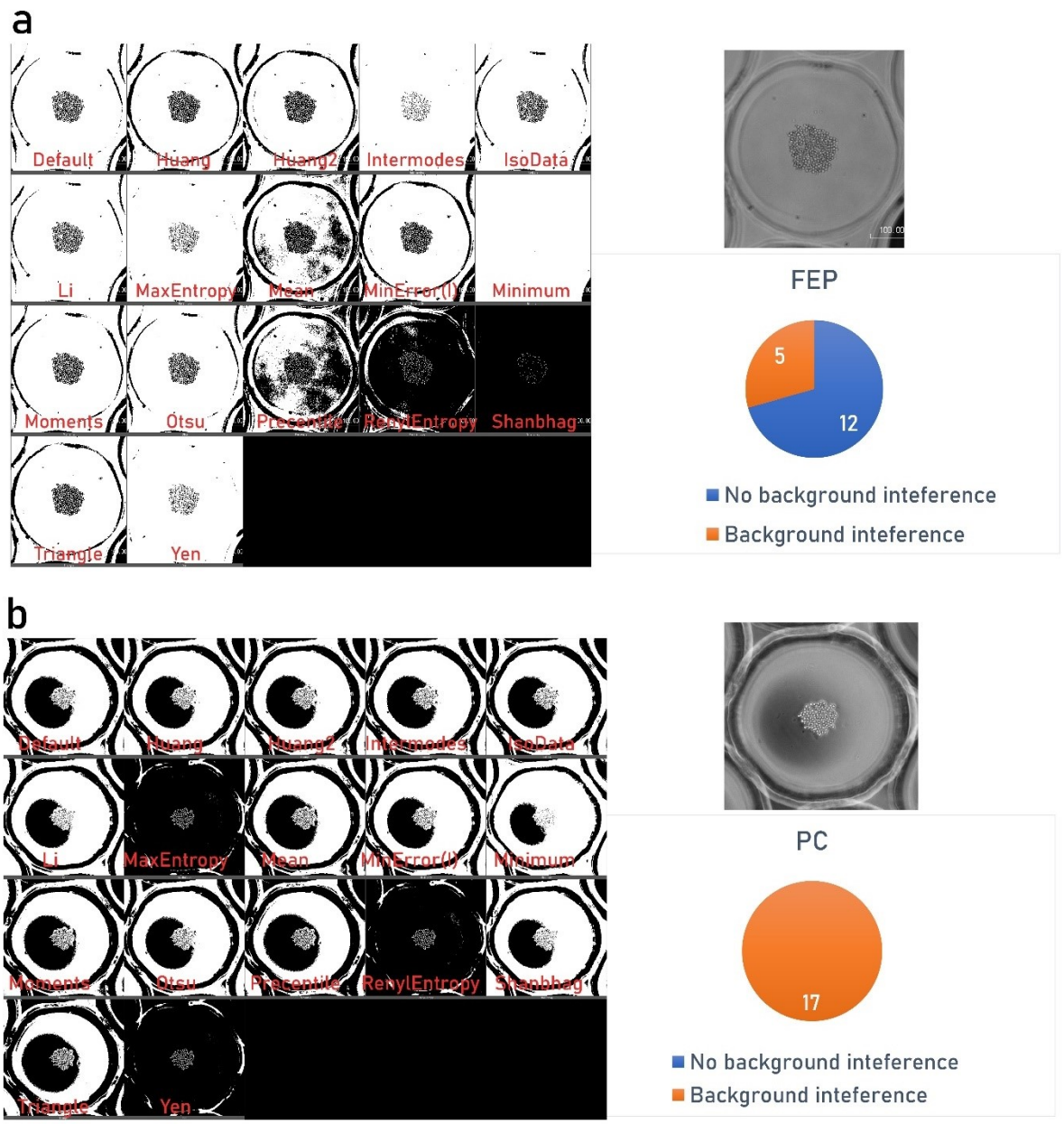


**Figure S6** Results of global image thresholding methods tested in FIJI performed on one randomly selected FEP and PC cavity (both of which had no visible imperfections like scratches, cracks or indents). a) Results for the FEP microcavity (left) and the corresponding input brightfield image showing the seeded mESCs on the right. Out of the 17 methods tested, 12 methods produced images where the cells did not have an overlap with the background (bottom right). b) Results for the PC microcavity (left) and the corresponding input brightfield image showing the seeded mESCs on the right. Out of the 17 methods tested, none of the tested methods produced images where the cells did not have an overlap with the background (bottom right). Both the FEP and PC microcavities were fabricated using a 800 µm diameter mold. The images were acquired using a Nikon eclipse TS100 inverted (manual) microscope in brightfield mode using Nikon DS-Fi1c-L3 camera. The images of the FEP and PC microcavity were obtained at 1/7 second and 1/10 second exposure time, respectively.

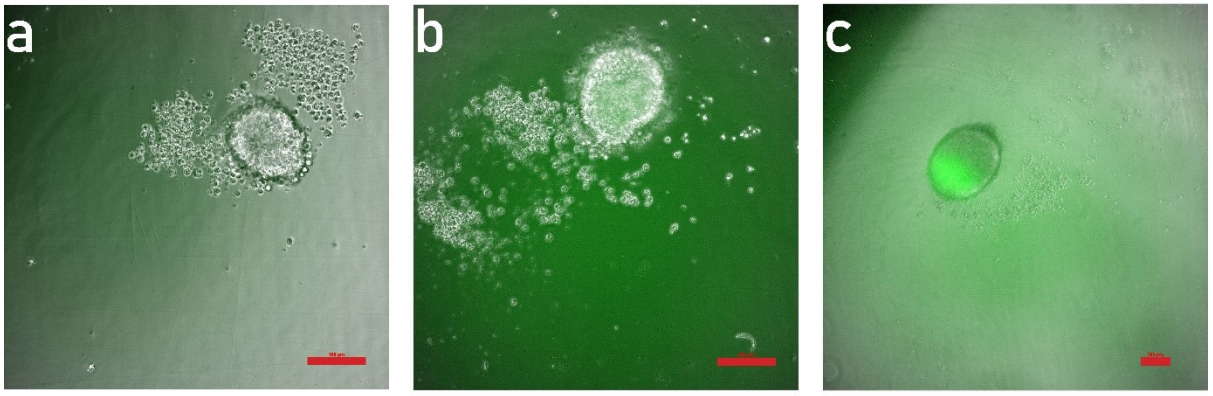


**Figure S7** Images of aggregates from Bra::GFP mESCs cultured in polystyrene U-bottomed 96 well plates (Greiner Cellstar 650185) as per the standard protocol for gastruloid culture[1] and imaged at a) 48 hps b) 72 hps and c) 96 hps. The Brachyury expression starts to appear at 72 hps and a polarized expression is observed at 96 hps along with aggregate elongation. All scale bars represent 100 µm. The images were obtained in widefield fluorescence mode using a Nikon Ti microscope.

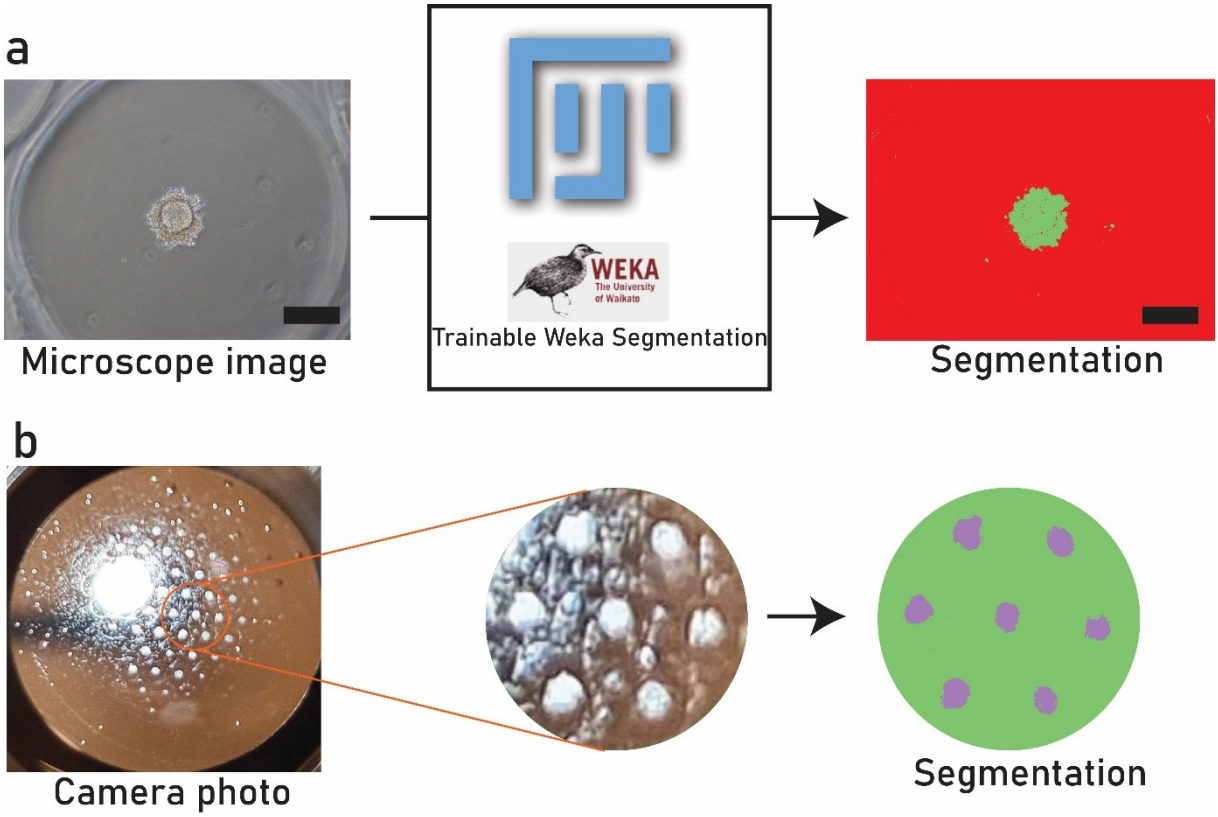


**Figure S8** Feature extraction using FIJI/TWS. a) Schematic showing the simplicity of the pipeline: the brightfield image is imported into FIJI where it is either used for training the machine learning model (or a pre-trained model is applied to it) generate the segmented image. b) The pipeline can also be applied to photos of the FEP microcavity array obtained from a mobile phone camera, which give rise to a segmented image showing the location and relative sizes of the cell clusters. Scale bar represents 100 µm.

ESI movie S1: Time-lapse video of Bra::GFP mESCs when cultured in FEP microcavities, obtained between 72 and 91.5 hps. The Brachyury signal is shown in green.

1. P. Baillie-Johnson, S. C. van den Brink, T. Balayo, D. A. Turner and A. Martinez Arias, 2015, DOI: doi:10.3791/53252, e53252.