Image processing algorithm to identify structure of tumour spheroids with cell cycle labelling

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Algorithm 1 Image processing algorithm using MATLAB R2021a (Mathworks).

1: Adjust contrast and exposure and mask spheroid of image using `AdjustRawImages.mlapp`. Output is a 4-layer `tif` file with the following channels:

> `raw(:,:,1)` (not used)
>
> `raw(:,:,2)` FUCCI green (Fig. 1b), corresponding to cells in gap 2.
>
> `raw(:,:,3)` FUCCI red (Fig. 1a), corresponding to cells in gap 2.
>
> `raw(:,:,4)` (not used)

2: Identify cells.

- 1: Obtain composite image `all = raw(:,:,2) + raw(:,:,3)` (Fig. 1c).
- 2: Apply the texture filter `stdfilt` and adjust gamma (1.1) to boost the signal (Fig. 1d).
- 3: Binarize using `imbinarize` to obtain `msk` (Fig. 1e).

4: Identify spheroid and necrotic core.

- 1: Dilate and then erode `msk` using a disk with radius 8 pixels to fill in gaps between cells.
- 2: Remove unconnected regions with an area less than 10000 pixels from `msk`.
- 3: Obtain convex hull of `msk`, named `cvx`, and convex area, `Acvx` (Fig. 1f). The spheroid radius (in pixels) is given by $\sqrt{\texttt{Acvx}/\pi}$. Scale appropriately to obtain R_{spheriod}.
- 4: Obtain the mask of the necrotic core, `msk2` $=$ `msk1` $-$ `msk`, where `msk1` is `msk` with all holes filled (Fig. 1g). An equivalent necrotic core radius (in pixels) is given by $\sqrt{\texttt{Amsk2}/\pi}$ where `Amsk2` is the area of `msk2`. Scale appropriately to obtain R_{necrotic}.

5: Obtain green pixel intensity distribution.

- 1: For each pixel in `cvx`, calculate the distance to the edge (nearest zero) in `cvx`, d_i, and the intensity of the FUCCI green channel `raw(:,:,2)`, g_i (Fig. 1h).
- 2: Calculate relative pixel intensity, $I(r)$, given by

$$I(r) = \frac{\sum_i \mathbf{1}(r - \Delta r/2 \leq d_i \leq r + \Delta r/2) \times g_i}{\sum_i \mathbf{1}(r - \Delta r/2 \leq d_i \leq r + \Delta r/2)}, \tag{1}$$

  where $\Delta r = \max(d_i)/100$ is the bin-width and $\mathbf{1}(\cdot)$ is an indicator function. We calculate $I(r)$ at 100 equally spaced points from Δr to $\max(d_i) - \Delta r$ (Fig. 1i).
- 3: Smooth observed intensity distribution by fitting a Gompertz function,

$$I_{\text{smooth}}(r) = p_1 \exp(-\exp(p_2(r - p_3))), \tag{2}$$

  to $I(r)$ using least squares (Fig. 1i).
- 4: If $I_{\text{smooth}}(\max(d_i)) > g_{\text{thresh}} I_{\text{smooth}}(0)$, there is no inhibited region. Else, calculate the distance from the periphery, r_{crit}, implicitly defined by $I_{\text{smooth}}(r_{\text{crit}}) = g_{\text{thresh}} I_{\text{smooth}}(0)$. The inhibited radius is given by $R_{\text{inhibited}} = R_{\text{spheriod}} - g_{\text{thresh}}$. Here, $g_{\text{thresh}} = 0.2$ is a tuneable threshold parameter.

6: Save results. The processed spheroid image is shown in Fig. 1j.

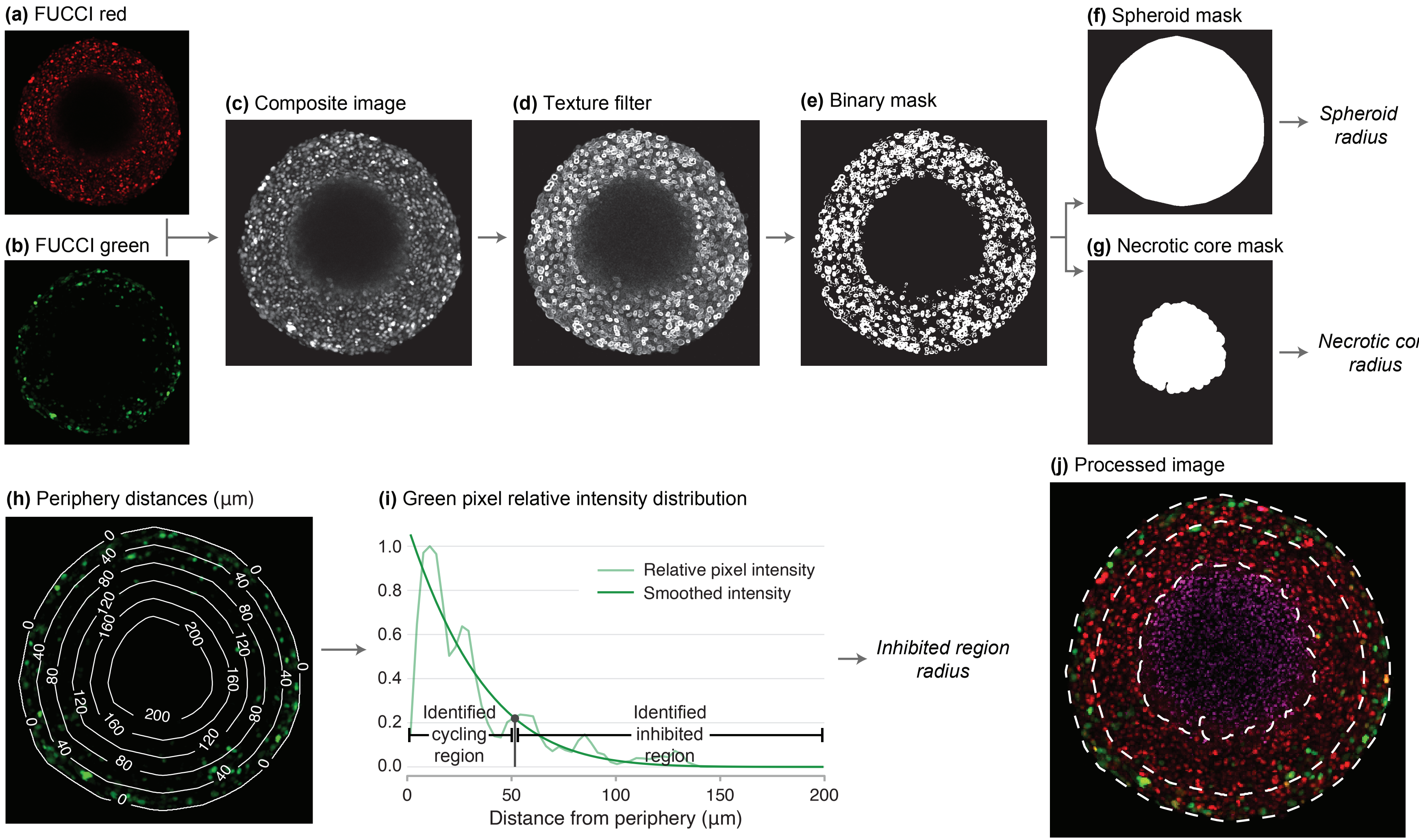
(a) FUCCI red
(b) FUCCI green
(c) Composite image
(d) Texture filter
(e) Binary mask
(f) Spheroid mask
Spheroid radius
(g) Necrotic core mask
Necrotic core radius
(h) Periphery distances (μm)
0
40
80
120
160
200
(i) Green pixel relative intensity distribution
1.0
0.8
0.6
0.4
0.2
0.0
Relative pixel intensity
Smoothed intensity
Identified cycling region
Identified inhibited region
0
50
100
150
200
Distance from periphery (μm)
Inhibited region radius
(j) Processed image

Figure 1