Developing a Jupyter Notebook for converting confocal microscopic images to 3D cell images and metrics calculation.

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3D segmentation

`do_3D=True` is the simplest way of 3D processing.

“If the 3D segmentation is not working well and there is inhomogeneity in Z, try stitching masks in Z”

Parameters to adjust:
- `cellprob_threshold`
- `flow_threshold`
- `anisotropy`
- `stitch_threshold`

- all pixels with value above threshold kept for masks, decrease to find more and larger masks

• Cellprob range: -6, 0, 6
• Fixed parameters: Flow_1.0, Stitch_1.4, Ani_120
flow_threshold

- flow error threshold (all cells with errors below threshold are kept) (not used for 3D)

• Flow range: 0.0, 0.5, 1.0 (default 0.4)
• Fixed parameters: Cellprob_-6, Stitch_1.4, Ani_120
anisotropy

- for 3D segmentation, optional rescaling factor (e.g. set to 2.0 if Z is sampled half as dense as X or Y)

• Anisotropy range: 50, 70, 90
  # I need to show changes in 3D, kinda 3 stacks. But these stacks have “white cells”
• Fixed parameters: Flow_1.0, Cellprob_-6, Stitch_1.4
stitch_threshold

- if stitch_threshold > 0.0 and not do_3D and equal image sizes, masks are stitched in 3D to return volume segmentation

• Stitch range: 0.1, 0.6, 0.9  \_same here, white stacks

• Fixed parameters: Flow_0.4, Cellprob_0, Ani_100
cell recognition parameters

- Can be ran without “z-slices parameters”.
  (anisotropy=None, stitch_threshold=0.0)

I choose ~500 (cellprob = 0, flow = 0.4). “Number of cells” is an amount of unique values, taken from cellpose function.
setup for z-slices

<table>
<thead>
<tr>
<th>stitch</th>
<th>0</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1</th>
<th>1.2</th>
<th>1.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>734</td>
<td>2210</td>
<td>2553</td>
<td>3191</td>
<td>4624</td>
<td>6437</td>
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</tr>
</tbody>
</table>

anisotropy

Cellprob_0, Flow_0.4, Ani_100, Stitch_0.5
Challenges

• “White cells”
• 3D image – can be done with bipartite algorithm
Thank you for your attention!