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

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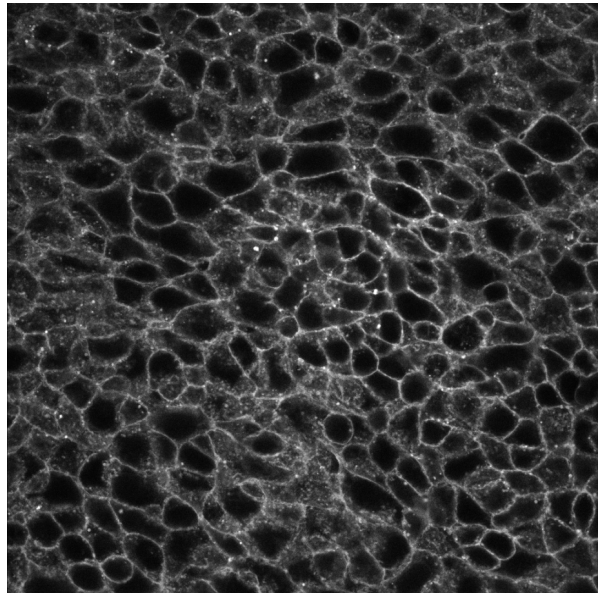
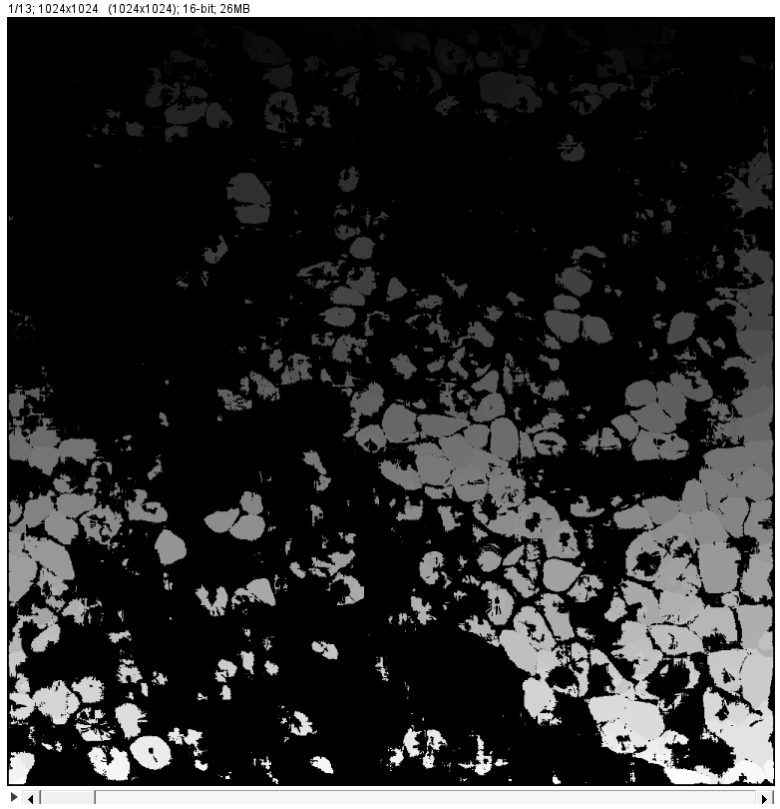
3D segmentation

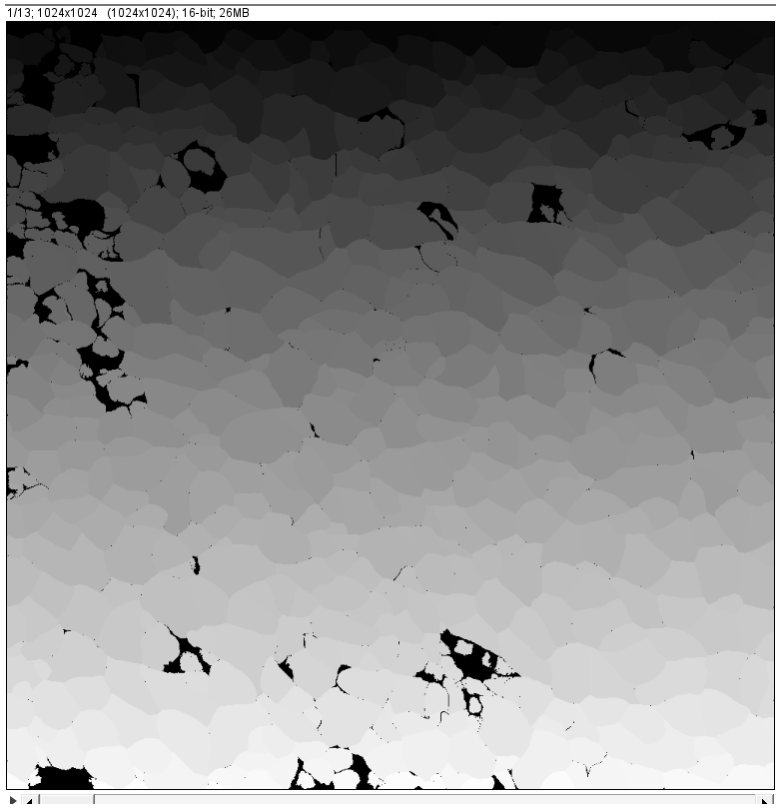
input image



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**

} cell recognition

- **anisotropy**
- **stitch_threshold**

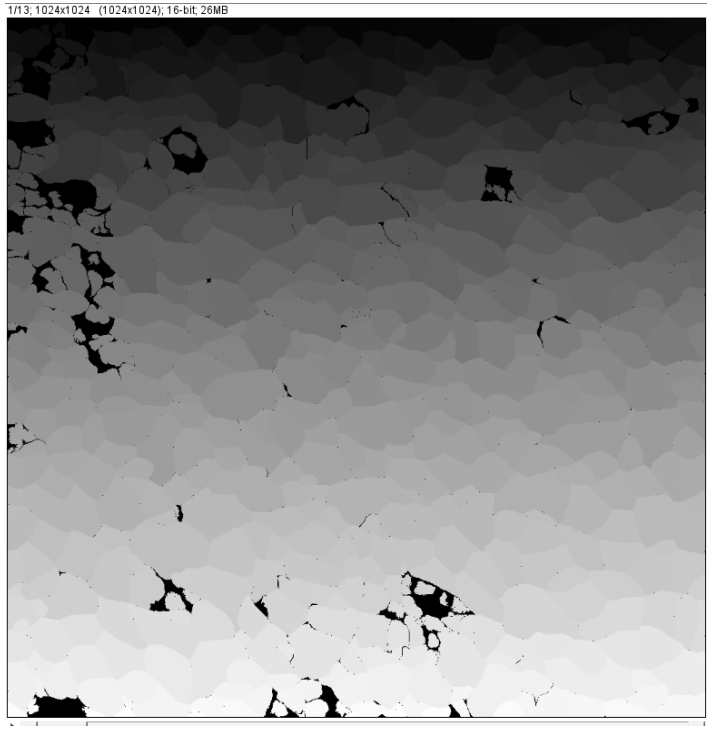
} setup for z-slices

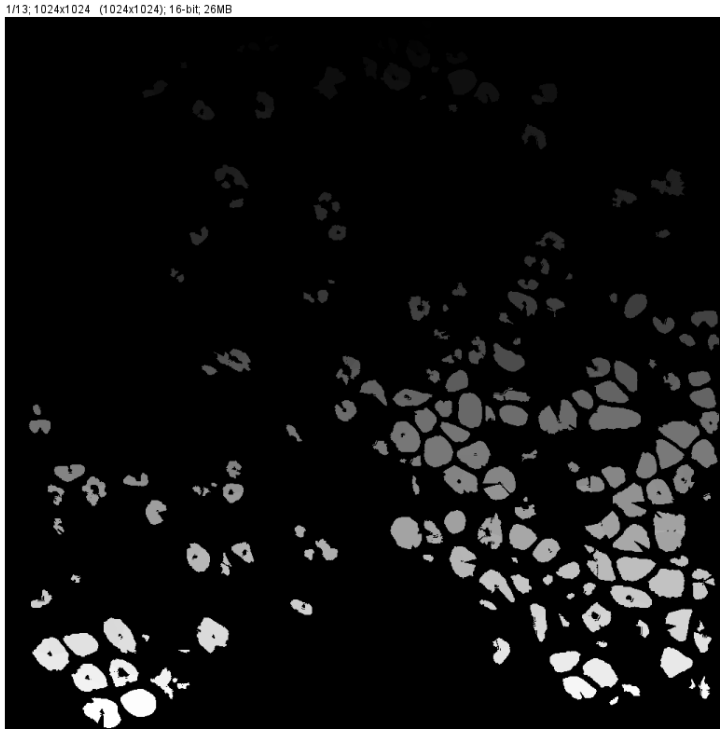
Python library – cellpose, "anatomical segmentation algorithm" - https://cellpose.readthedocs.io

cellprob_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

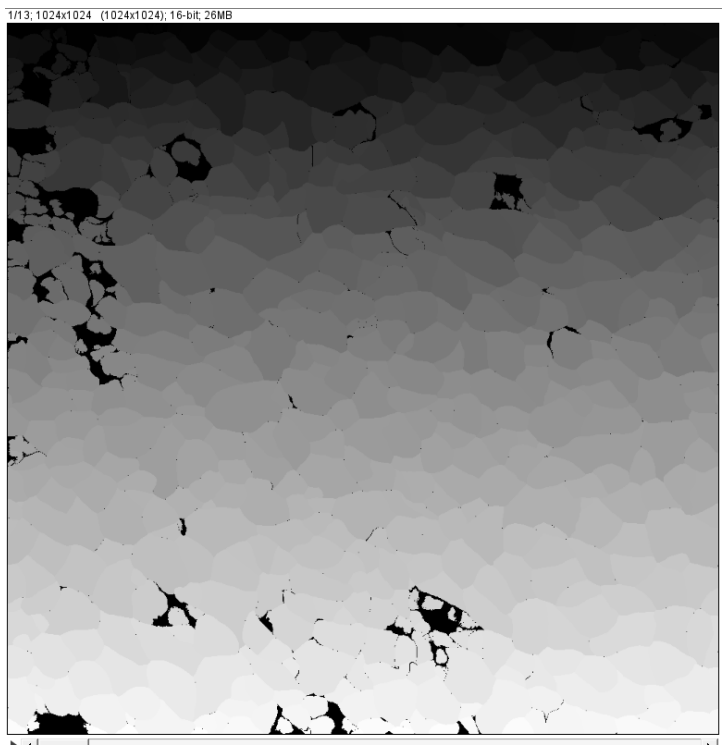


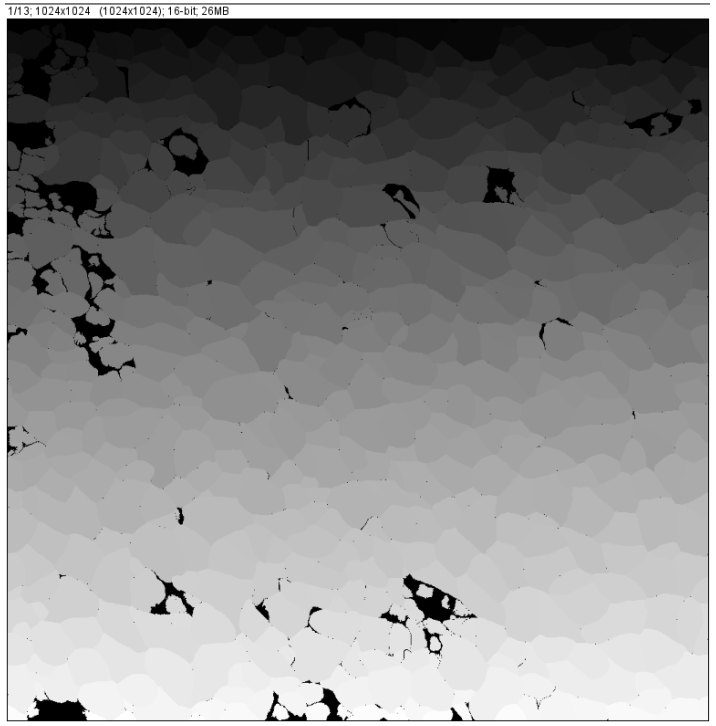


flow_threshold

- 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

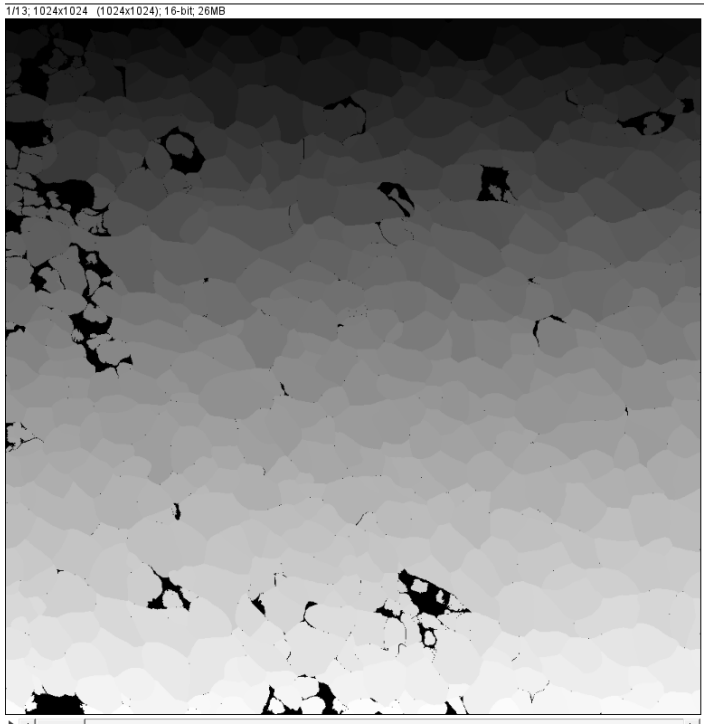


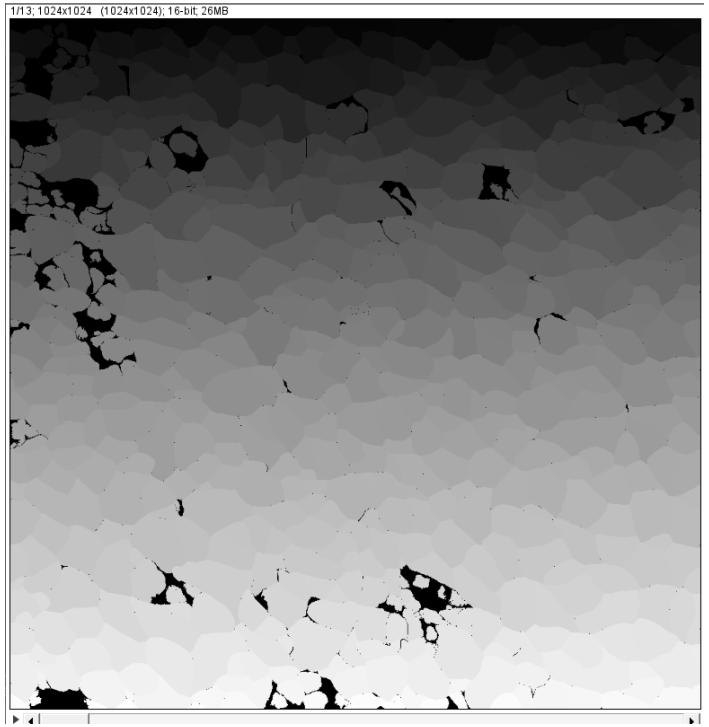


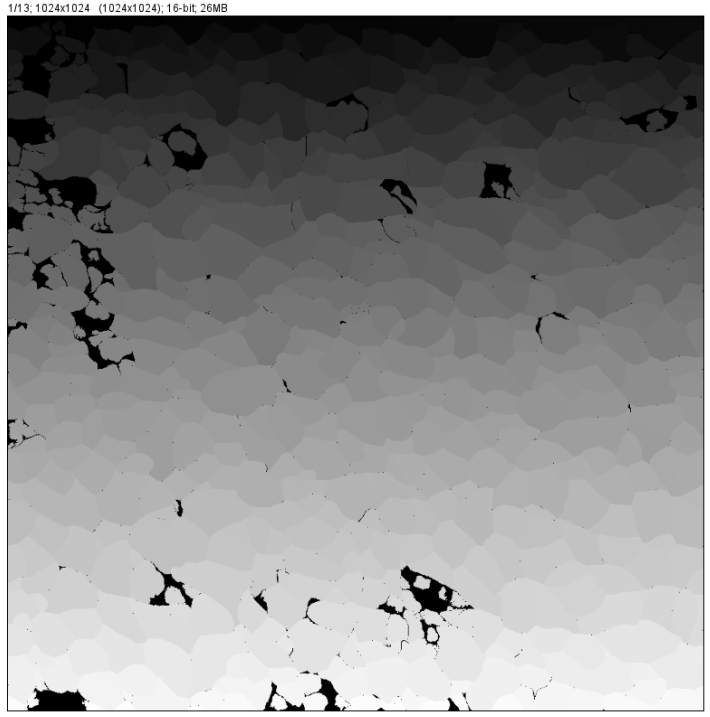
anisotropy

- 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



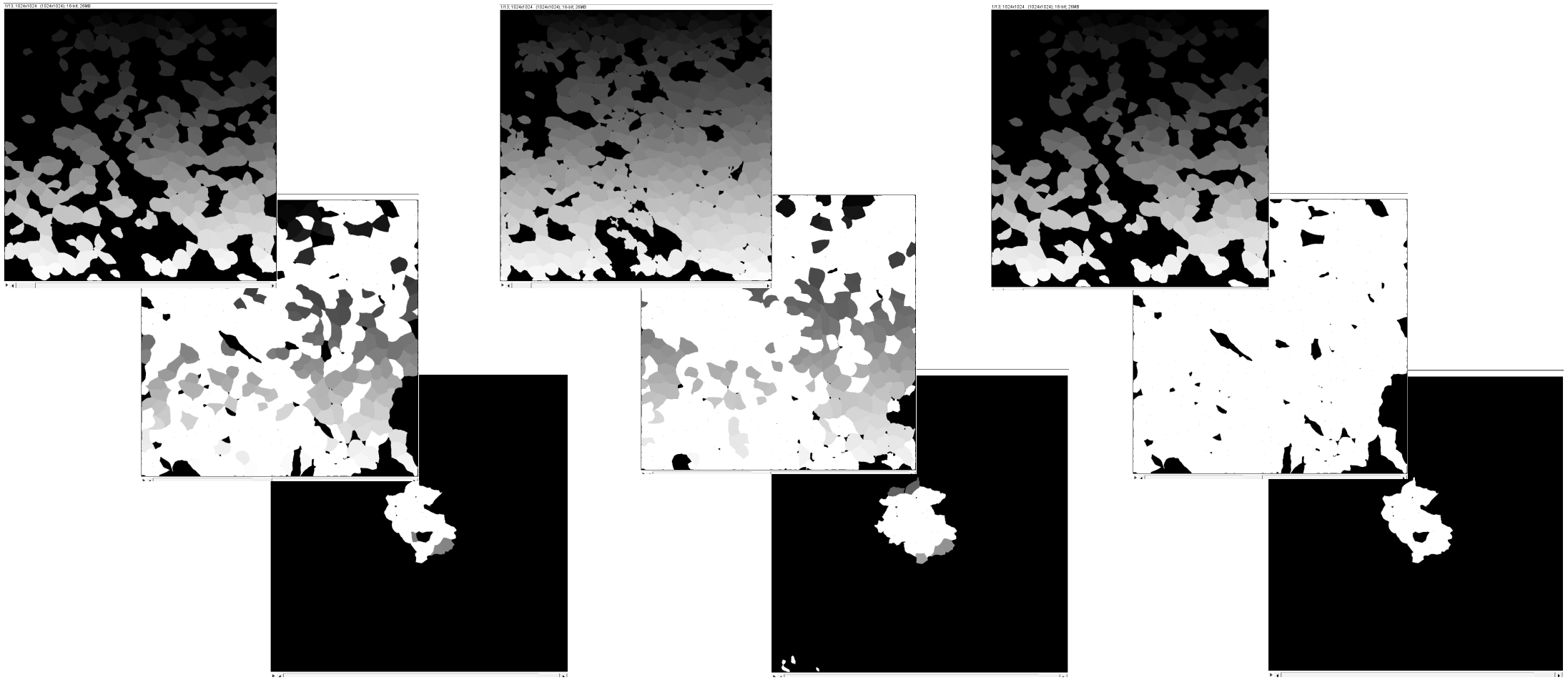




stitch_threshold

- 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)

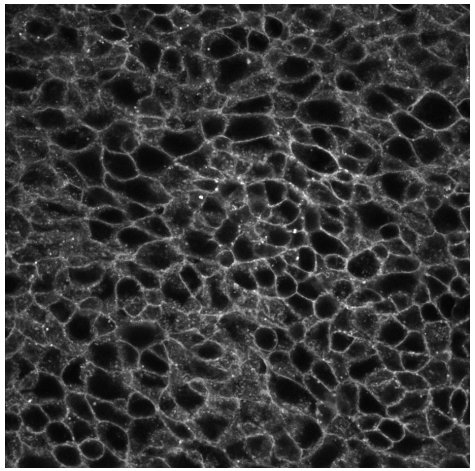
flow_threshold

cellprob_threshold

flow	0	0.2	0.4	0.6	0.8	1	1.2	1.4	1.6
-6	720	462	511	574	609	705	717	720	720
-4	734	462	511	574	609	718	731	734	734
-2	761	463	511	572	609	701	759	761	761
0	744	464	512	561	579	605	697	737	744
2	634	470	496	512	516	524	545	588	631
4	576	440	456	464	468	475	487	507	538
6	806	225	266	296	322	354	411	500	601

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

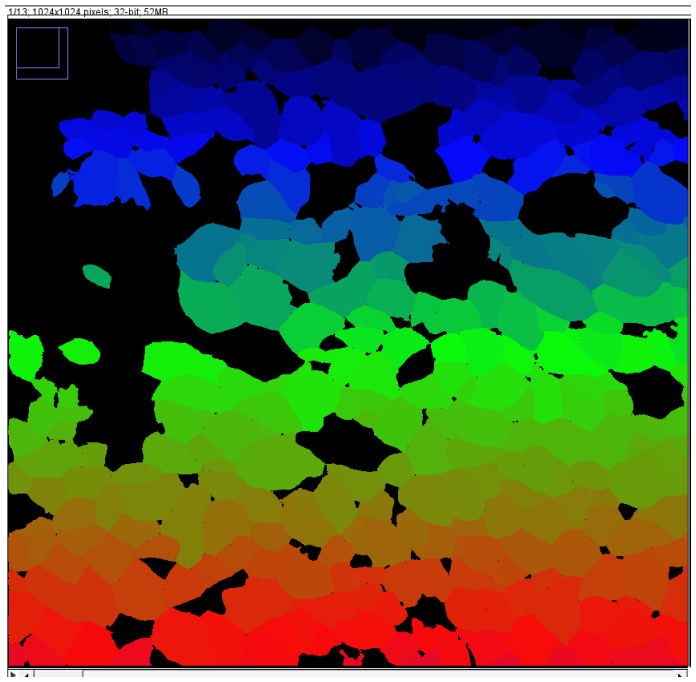


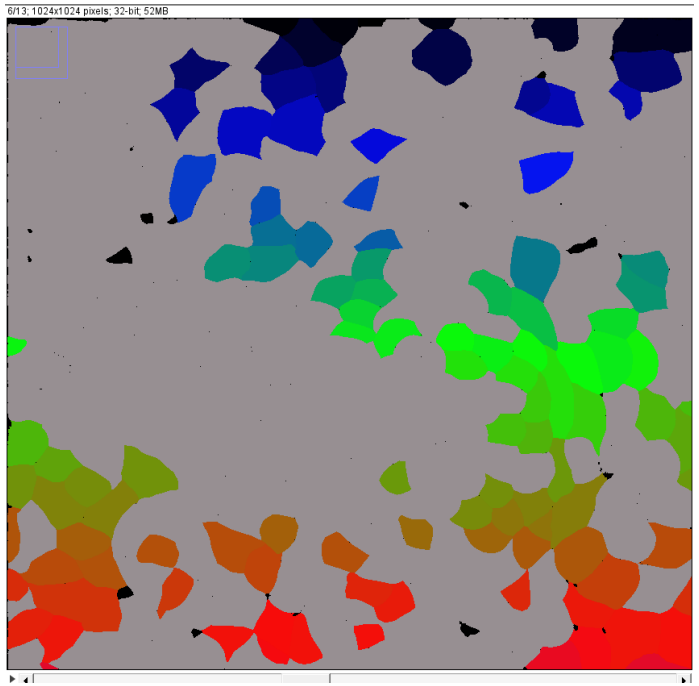
stitch_threshold

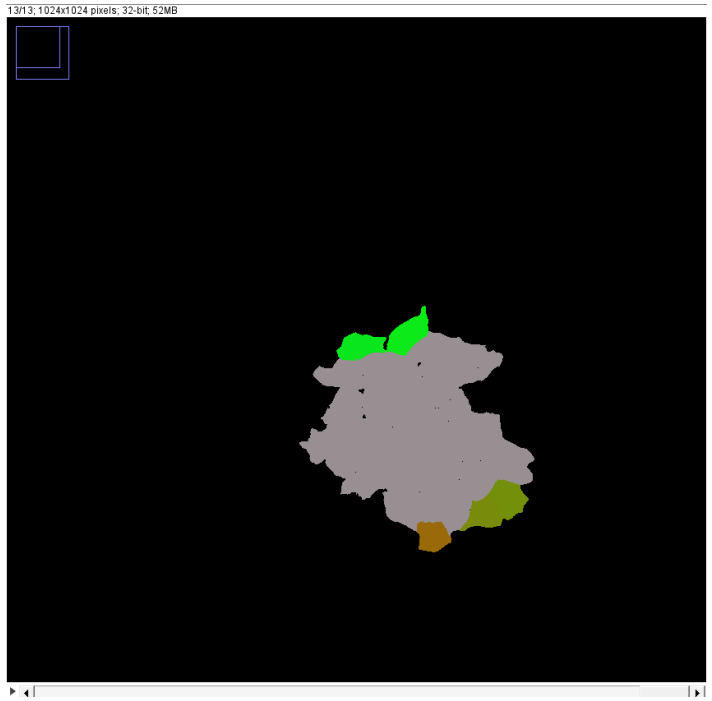
anisotropy

stitch	0	0.2	0.4	0.6	0.8	1	1.2	1.4
50	734	2210	2553	3191	4624	6437	6437	6437
70	734	2210	2553	3191	4624	6437	6437	6437
90	734	2210	2553	3191	4624	6437	6437	6437
110	734	2210	2553	3191	4624	6437	6437	6437
130	734	2210	2553	3191	4624	6437	6437	6437

Cellprob_0,
Flow_0.4,
Ani_100,
Stitch_0.5

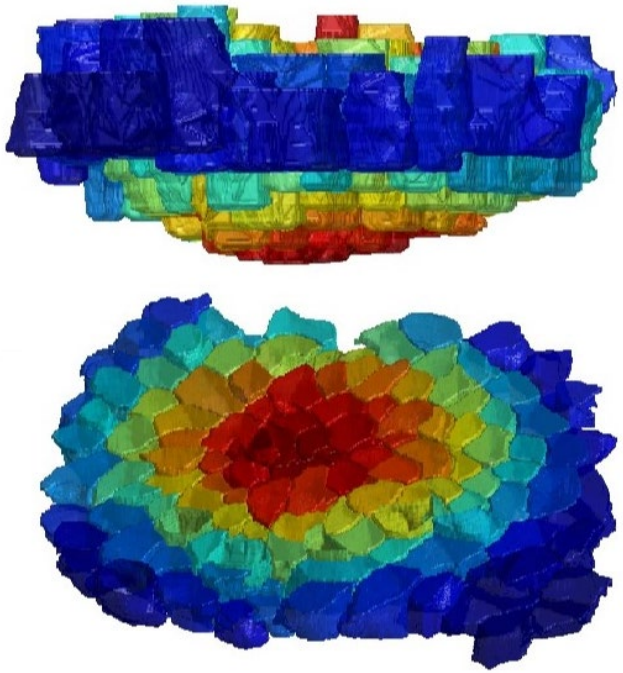






Challenges

- “White cells”
- 3D image – can be done with bipartite algorithm



Thank you for your attention!