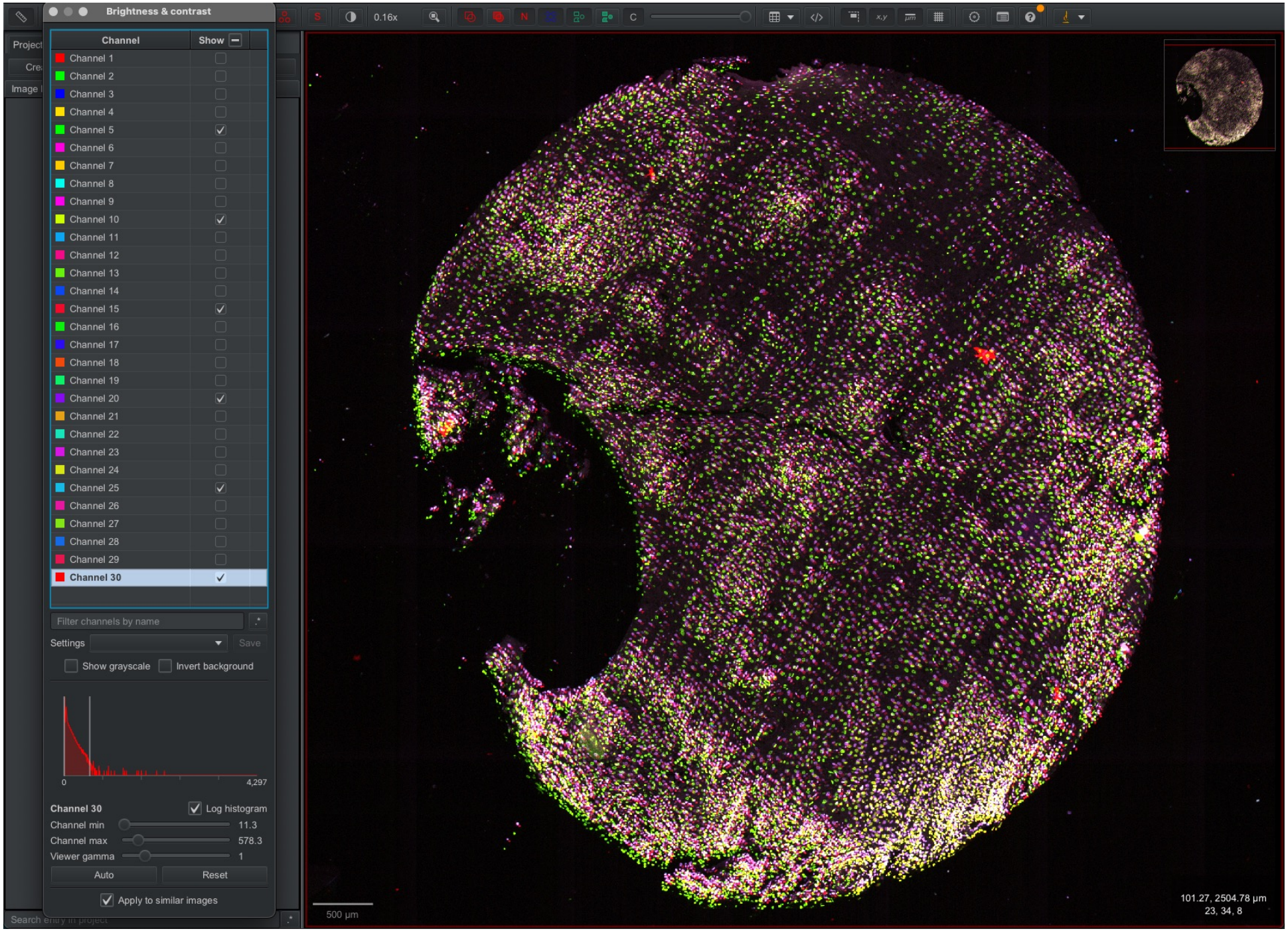
Stitching,
a story to understand it

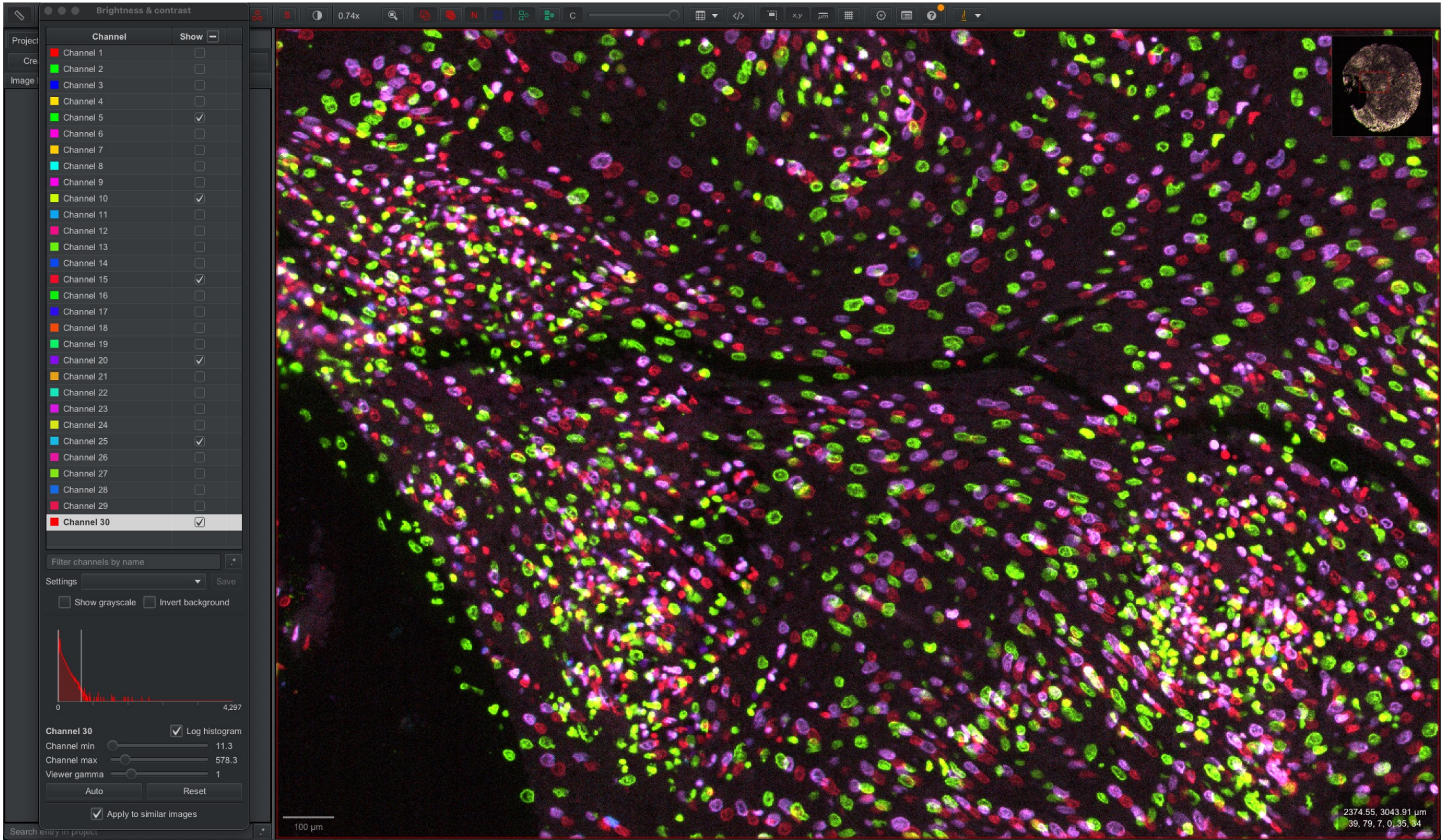
HN46 Core 5

Baseline:
Ashlar
stitching and registration

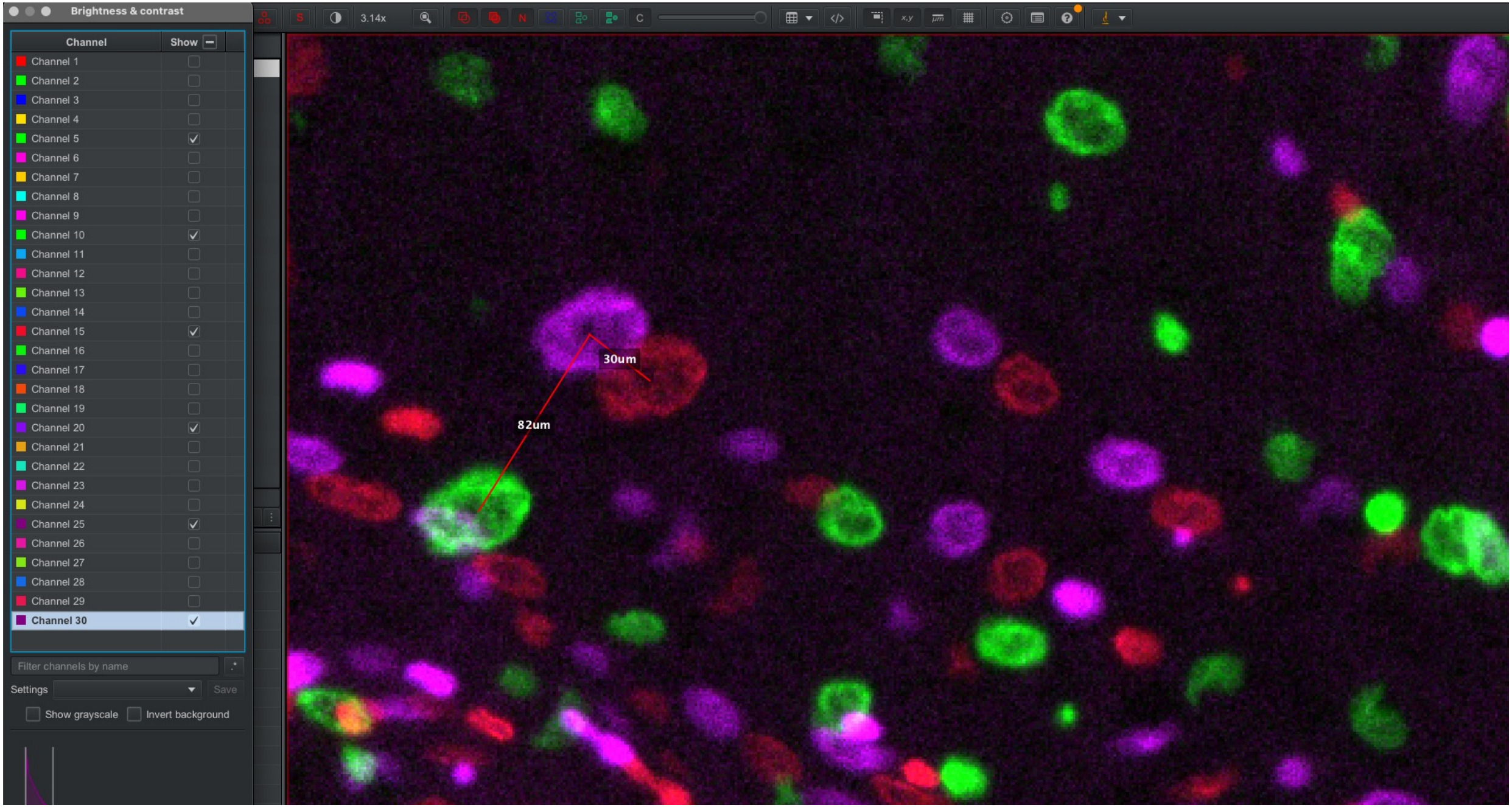
Parameters:
Max_shift: 20um
Sigma: 1



BASELINE


Brightness & contrast
Channel
Show
Channel 1
Channel 2
Channel 3
Channel 4
Channel 5
Channel 6
Channel 7
Channel 8
Channel 9
Channel 10
Channel 11
Channel 12
Channel 13
Channel 14
Channel 15
Channel 16
Channel 17
Channel 18
Channel 19
Channel 20
Channel 21
Channel 22
Channel 23
Channel 24
Channel 25
Channel 26
Channel 27
Channel 28
Channel 29
Channel 30
Filter channels by name
Settings
Save
Show grayscale
Invert background
0
4,297
Channel 30
Log histogram
Channel min
11.3
Channel max
578.3
Viewer gamma
1
Auto
Reset
Apply to similar images
0.74x
100 µm
2374.55, 3043.91 µm

BASELINE


Brightness & contrast
Channel
Show
Channel 1
Channel 2
Channel 3
Channel 4
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Channel 12
Channel 13
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Channel 18
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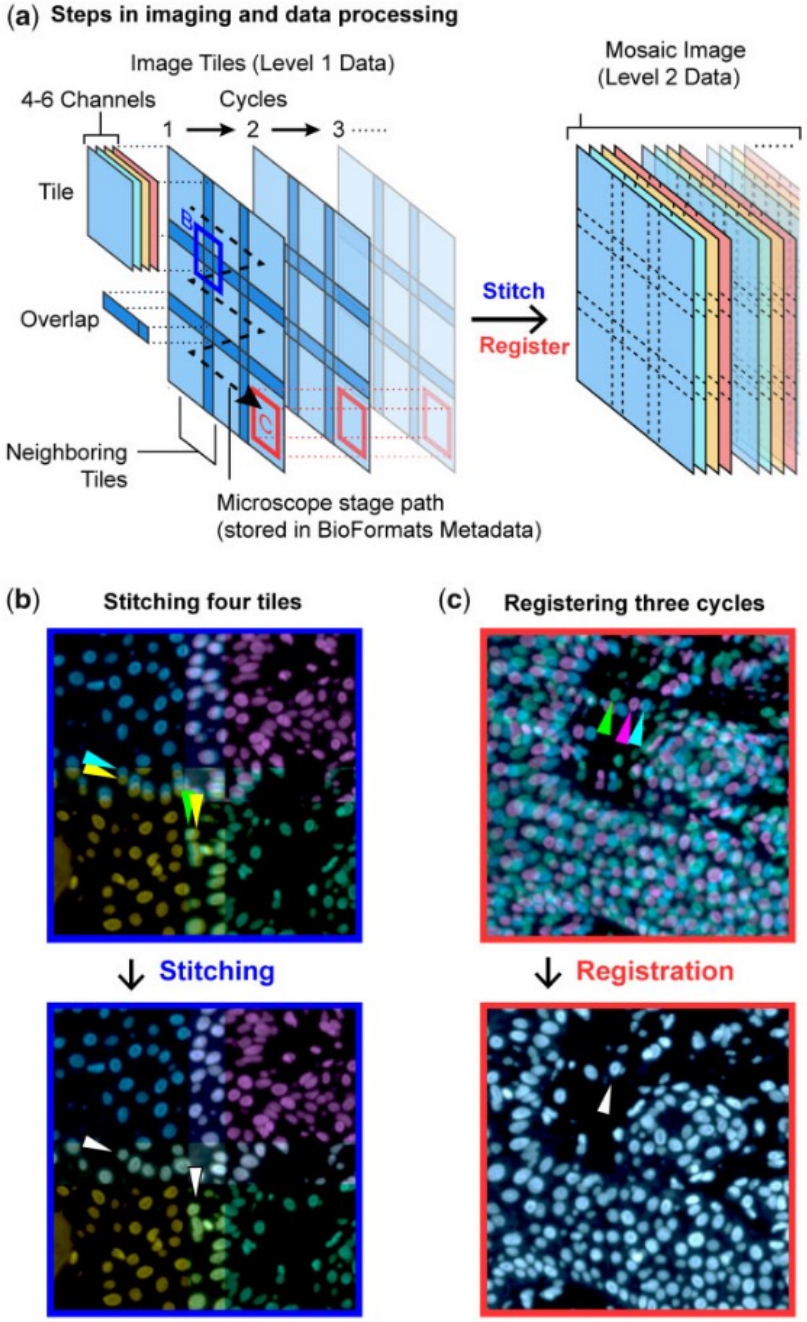
Channel 20
Channel 21
Channel 22
Channel 23
Channel 24
Channel 25
Channel 26
Channel 27
Channel 28
Channel 29
Channel 30
Filter channels by name
Settings
Save
Show grayscale
Invert background
3.14x
30um
82um

Not good enough

- BASELINE fails
- Stitching, registration, or both?

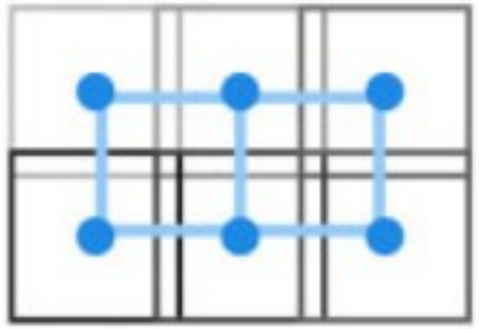
QC Metrics:

- Edge shift distance (tile shift)
- Edge Error probability (against MC)

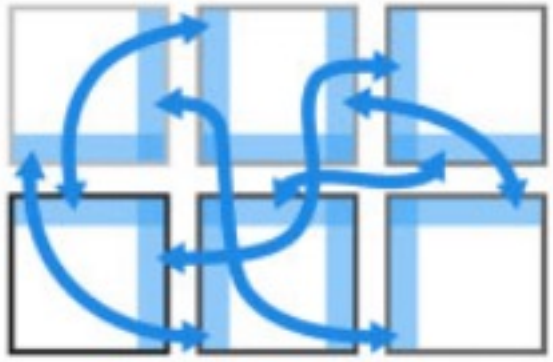


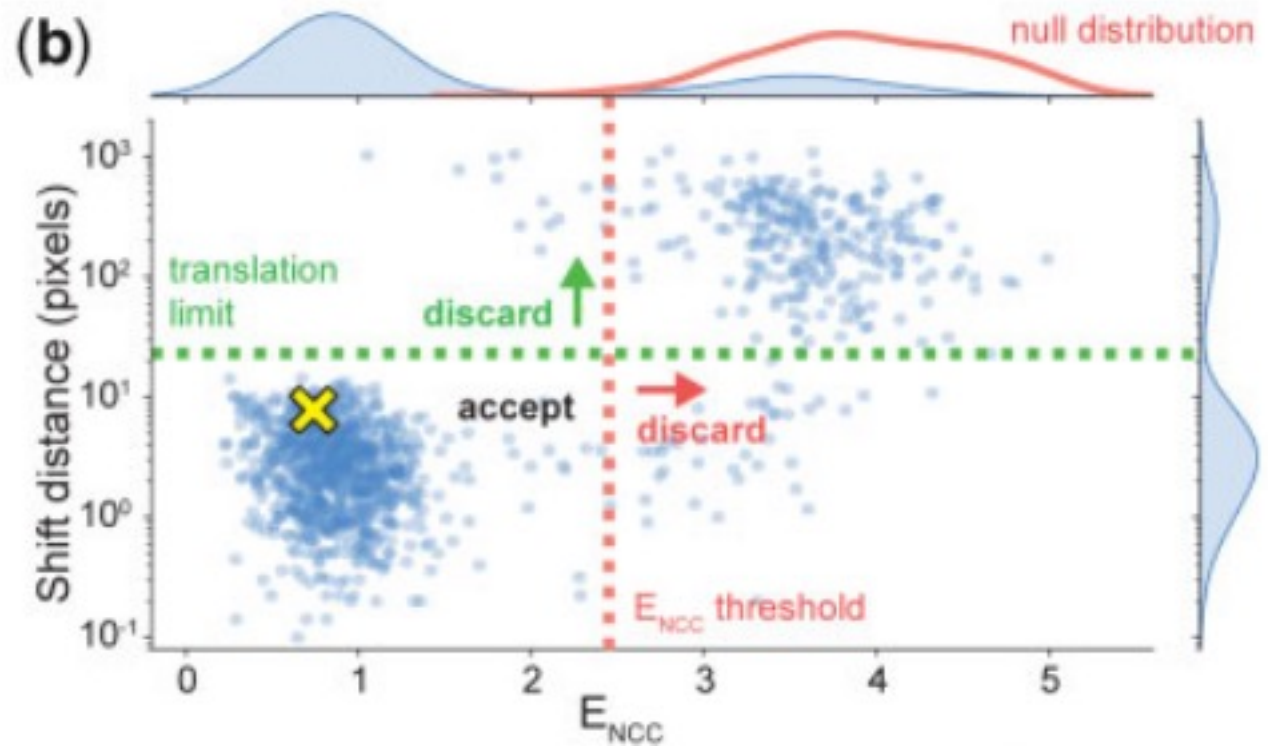
Stitching QC: what we want?

A1. Tile adjacency graph from stage positions

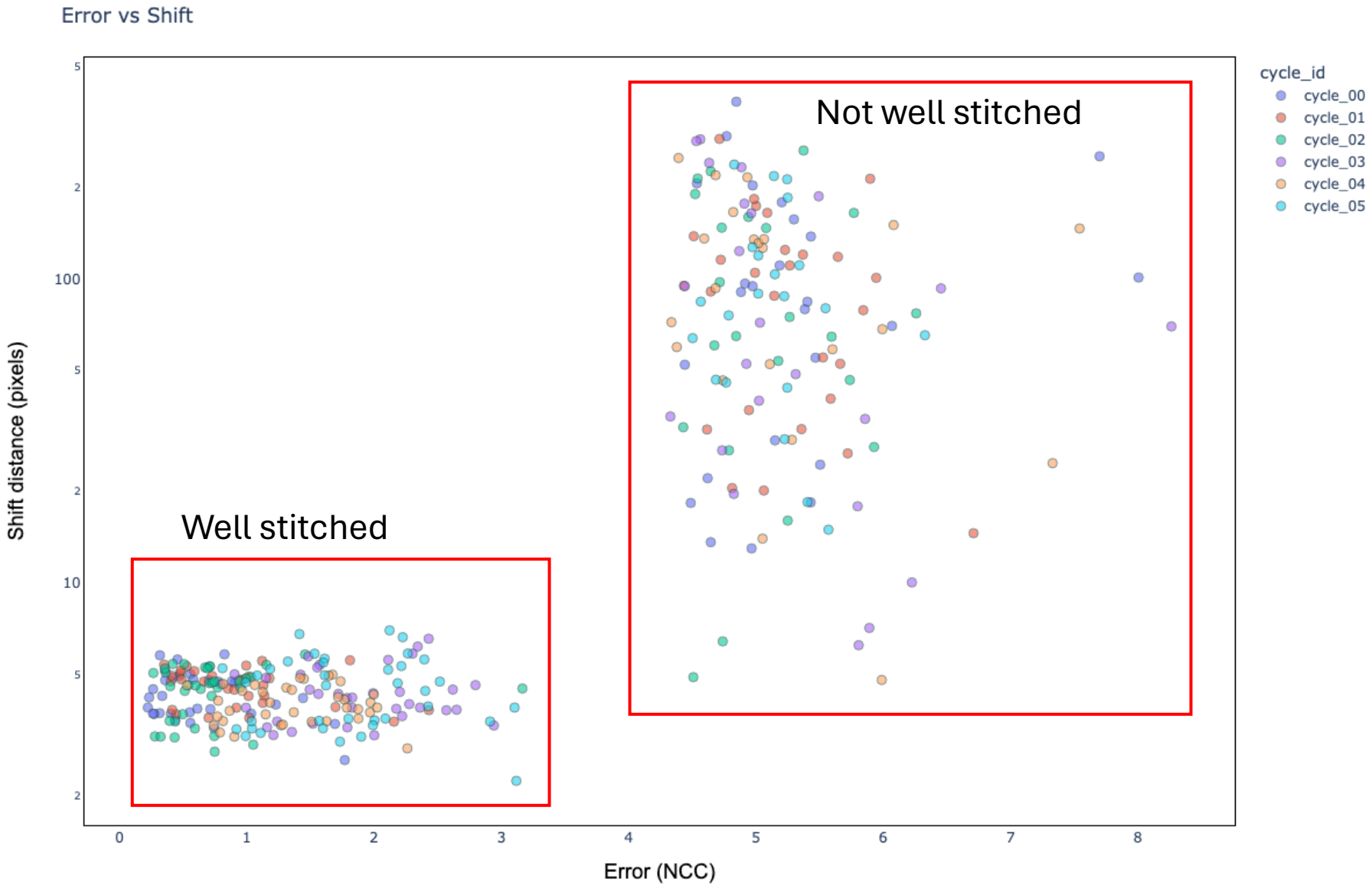


A2. Permutation test to find error threshold





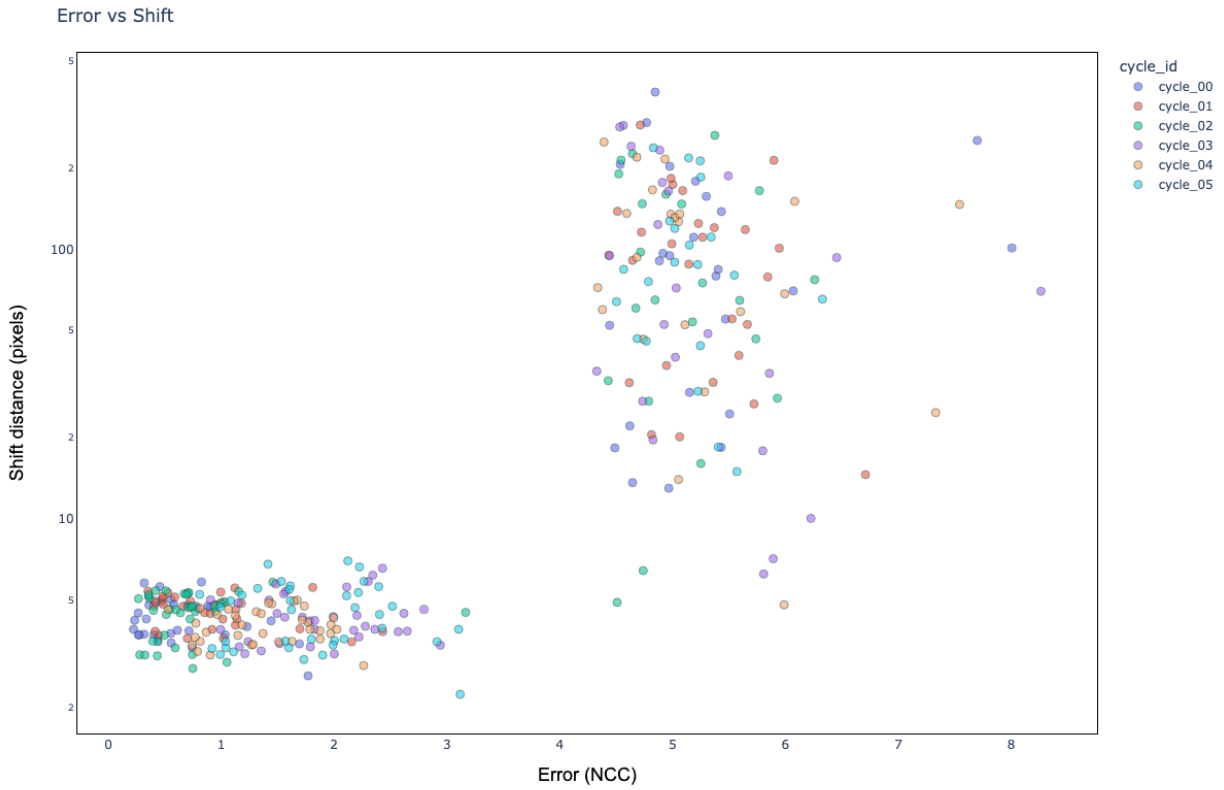
BASELINE:
Shift 25
Sigma 1

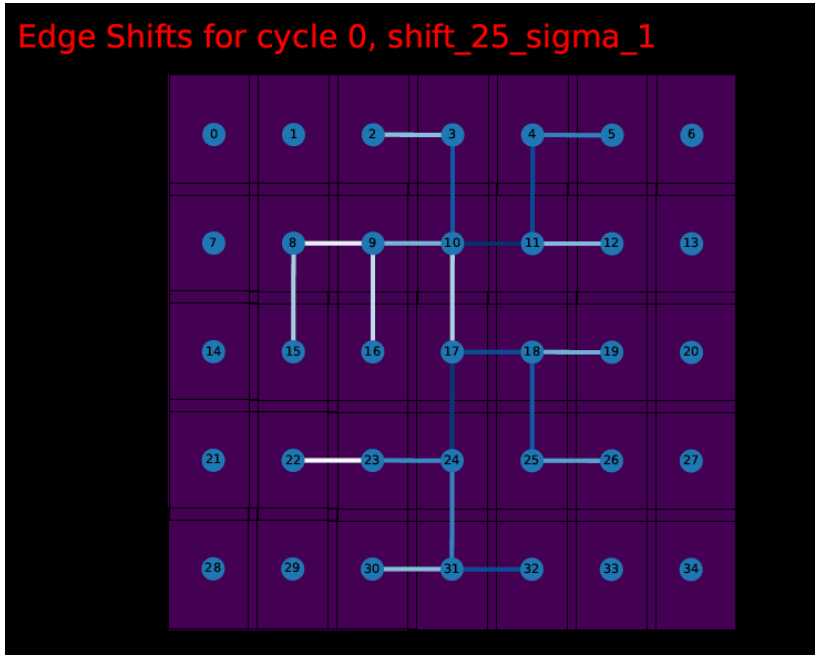


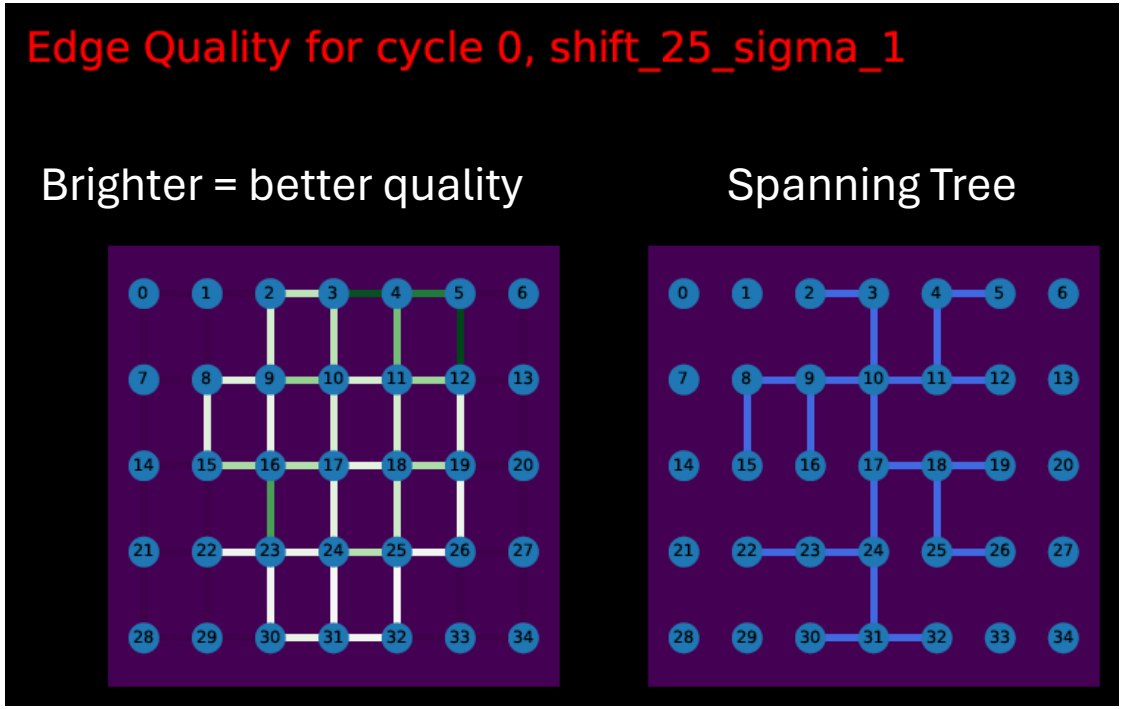
Ok,
but not good enough

Consider that we expect tiles surrounding the core to fail at stitching

BASELINE:
Shift 25
Sigma 1



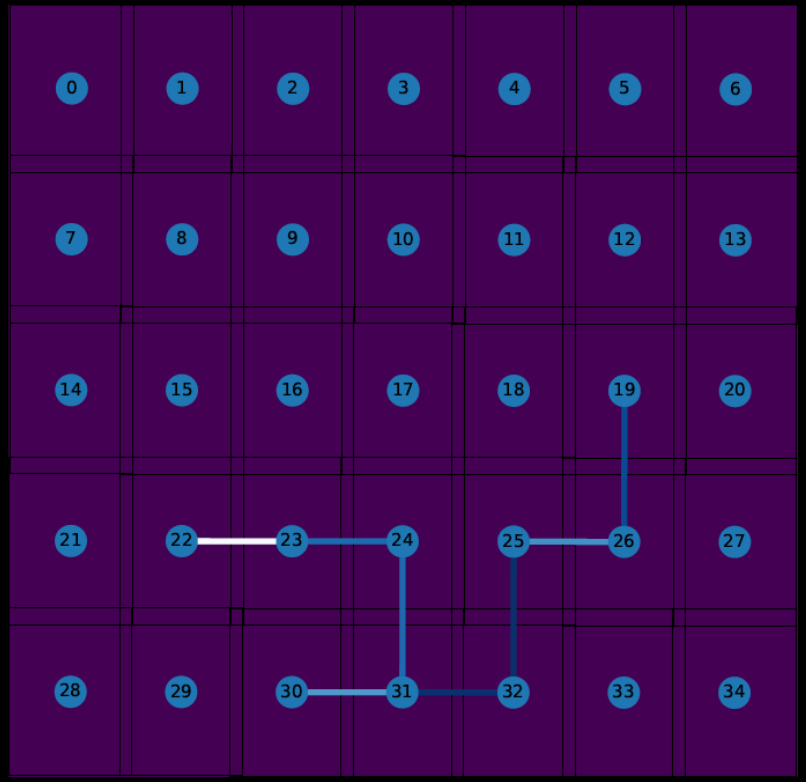




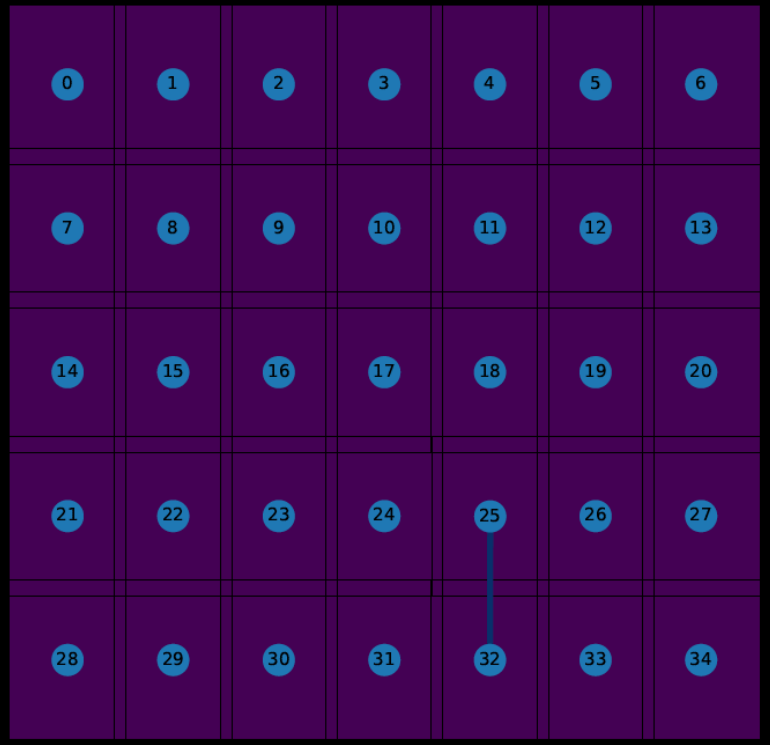
Testing sigma changes

Conclusion:
Sigma 0 does not work

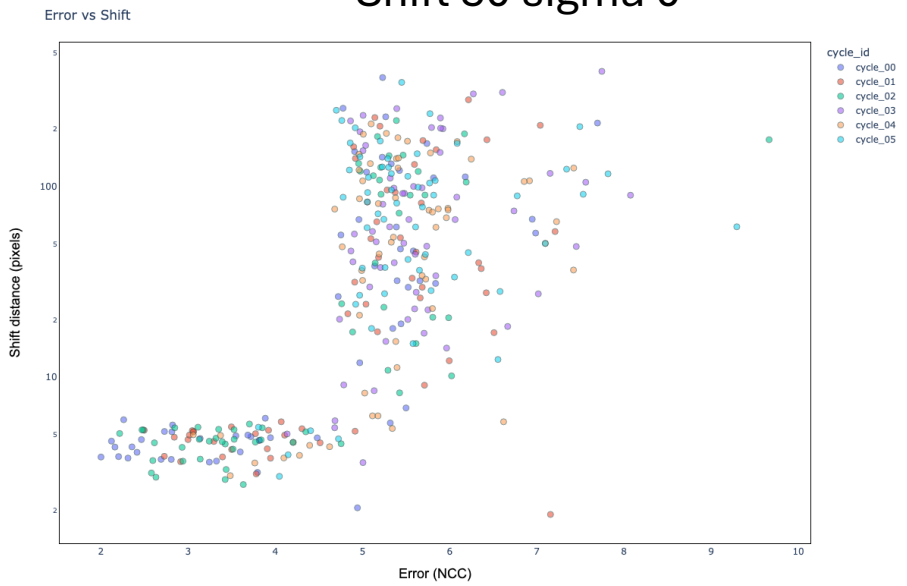
Edge Shifts for cycle 0, shift_25_sigma_0



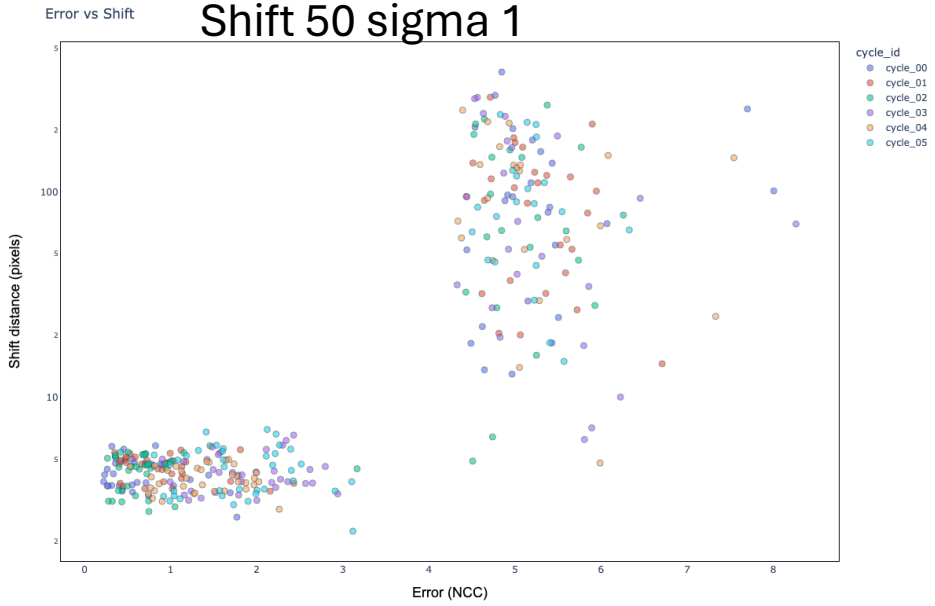
Edge Shifts for cycle 1, shift_25_sigma_0



Shift 50 sigma 0



Shift 50 sigma 1

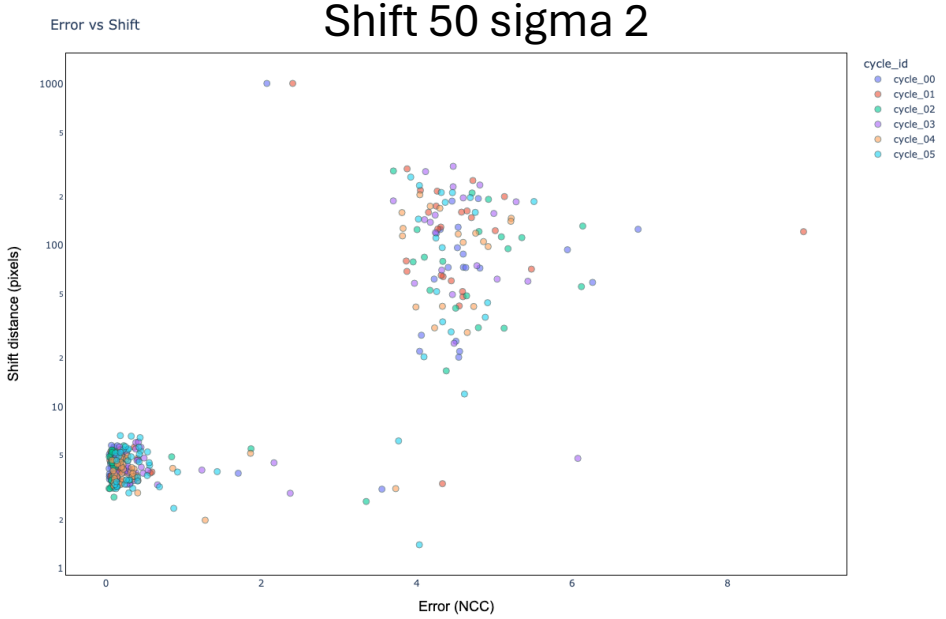


Question:
What about different sigmas?

Tested:
Sigmas 0,1,2,4

Conclusion:
=>1 seems to be good enough

Shift 50 sigma 2

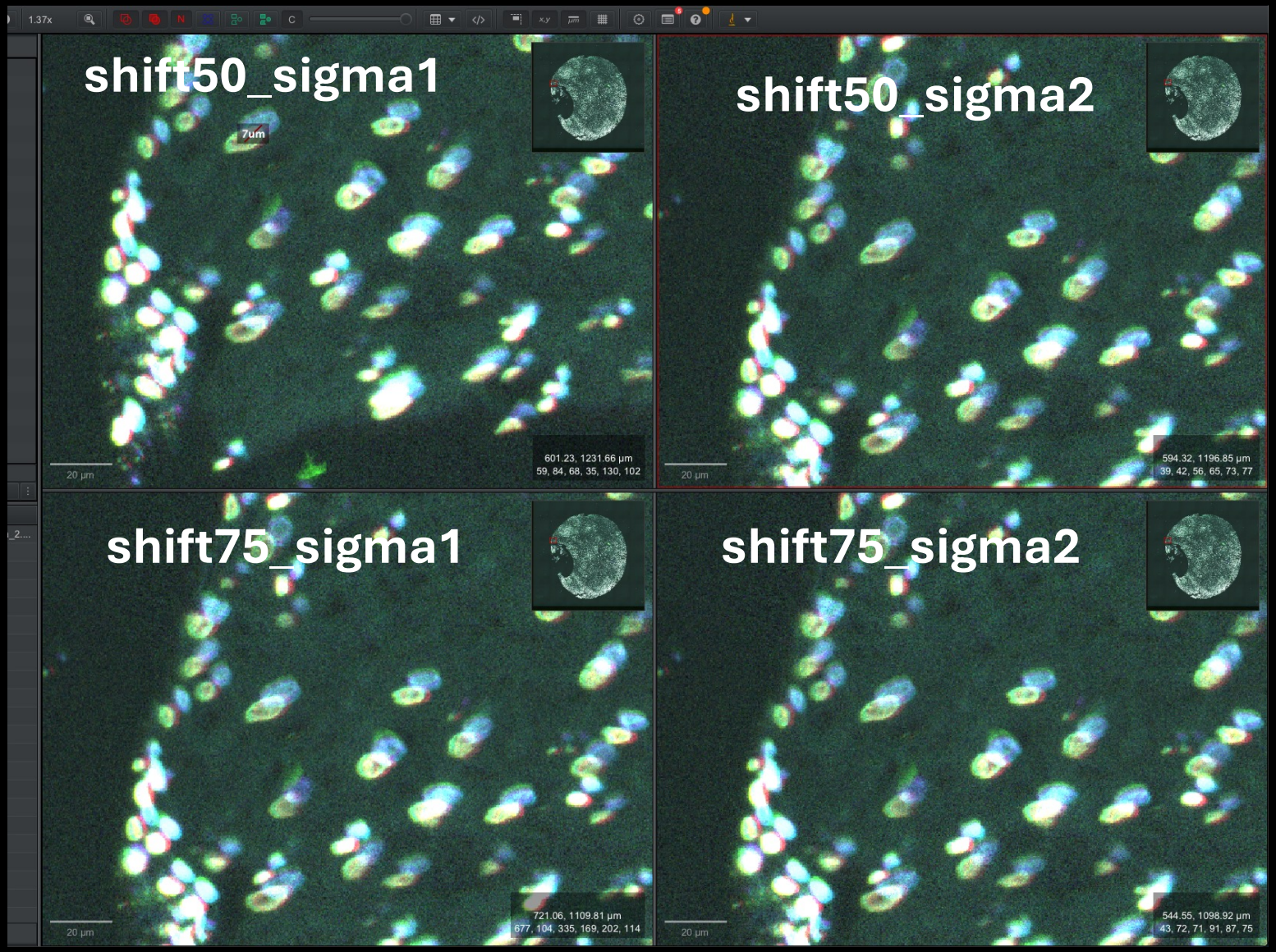


Shift 50 sigma 4

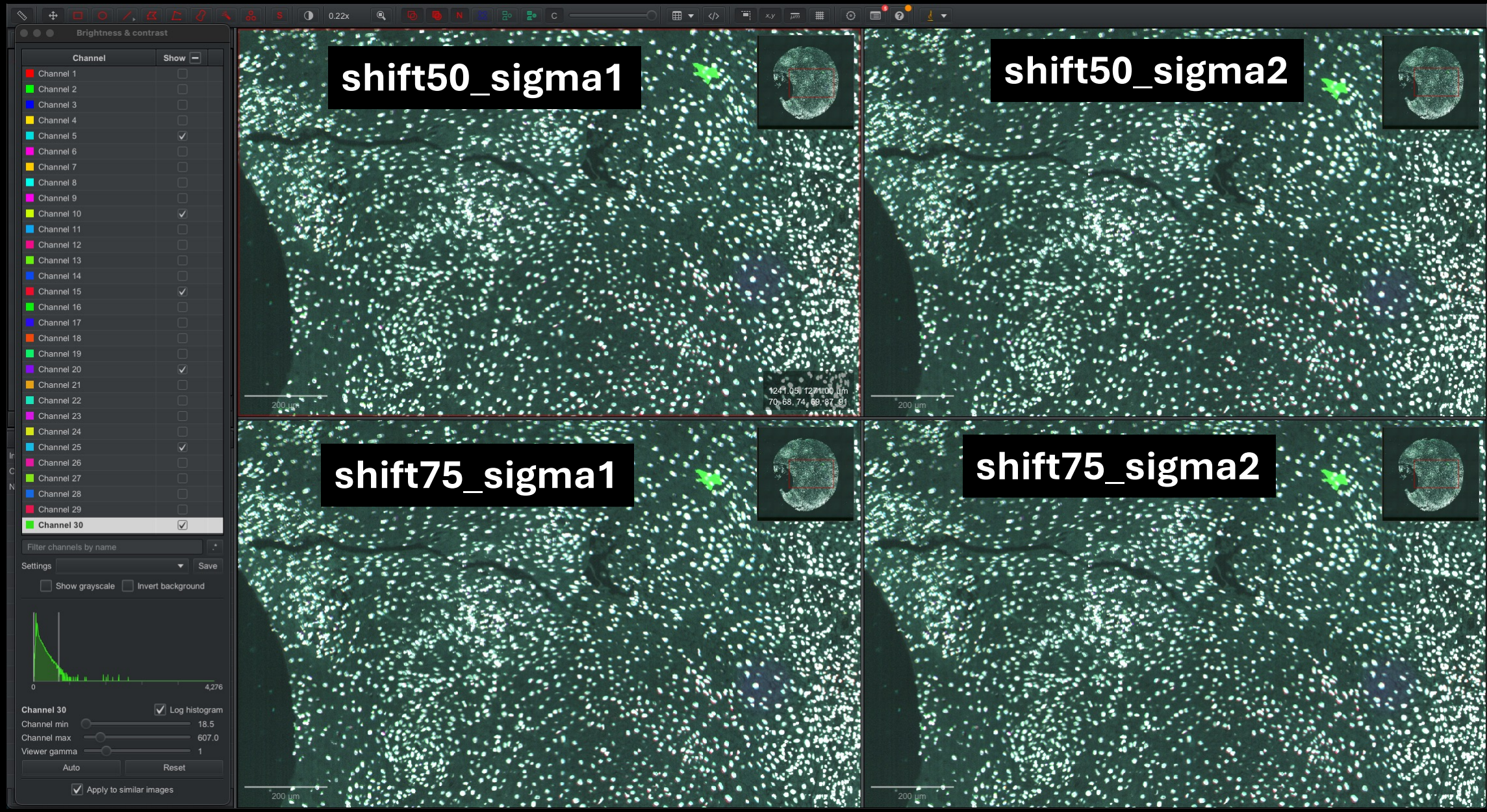
Question:
Shift sigma changes

Conclusion:
Shift must be at least 50
Sigma must be at least 1

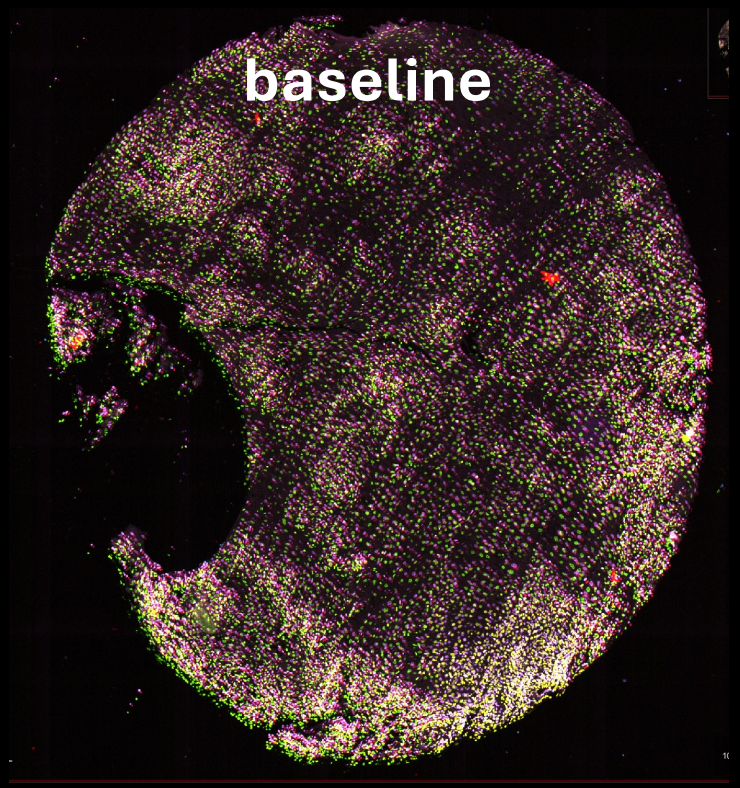
7um shift much better compared to 80/30um shifts seen before



Visualizing. Seems good, but still a bit blurry.

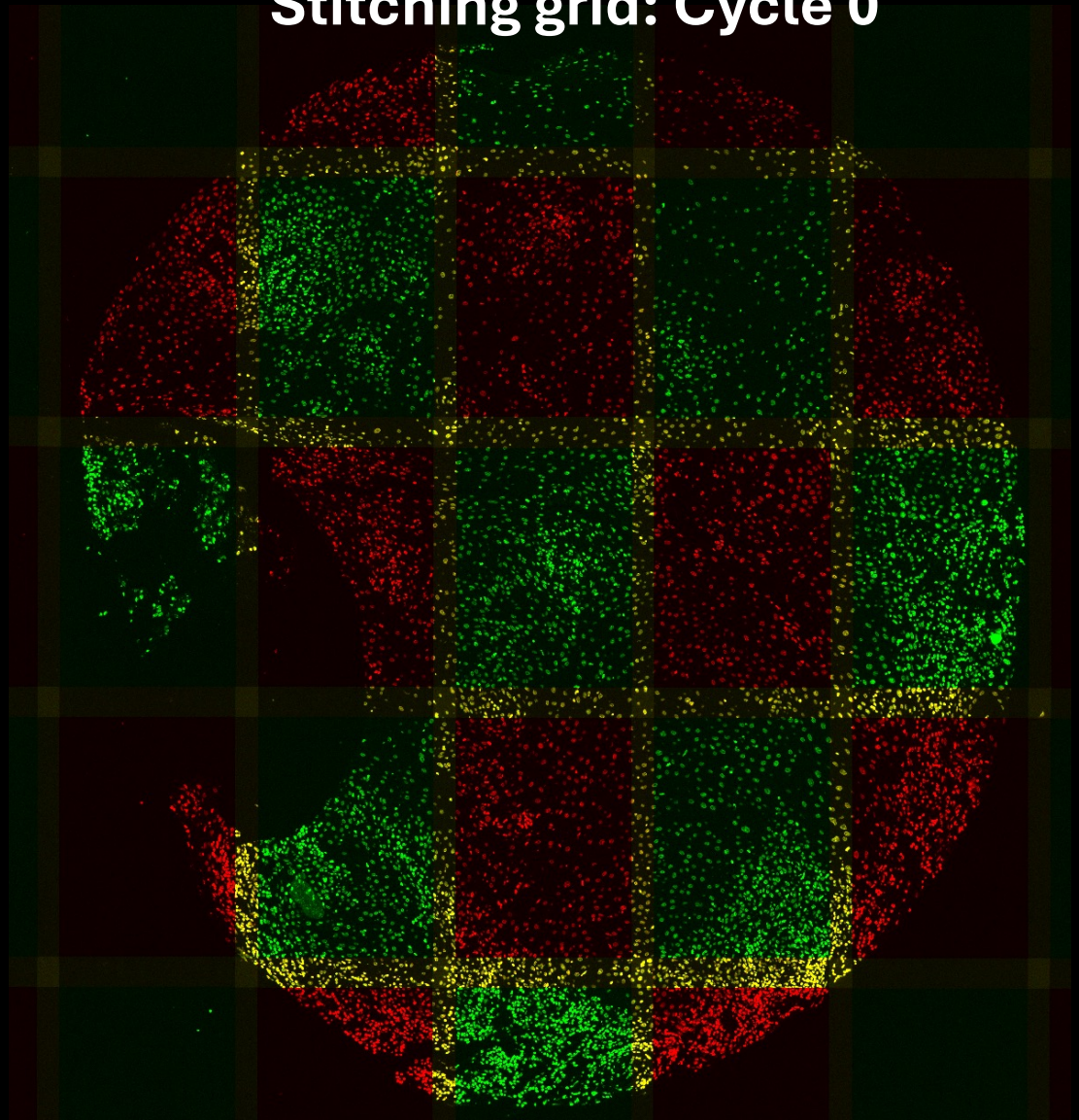


Visualizing. Still much better

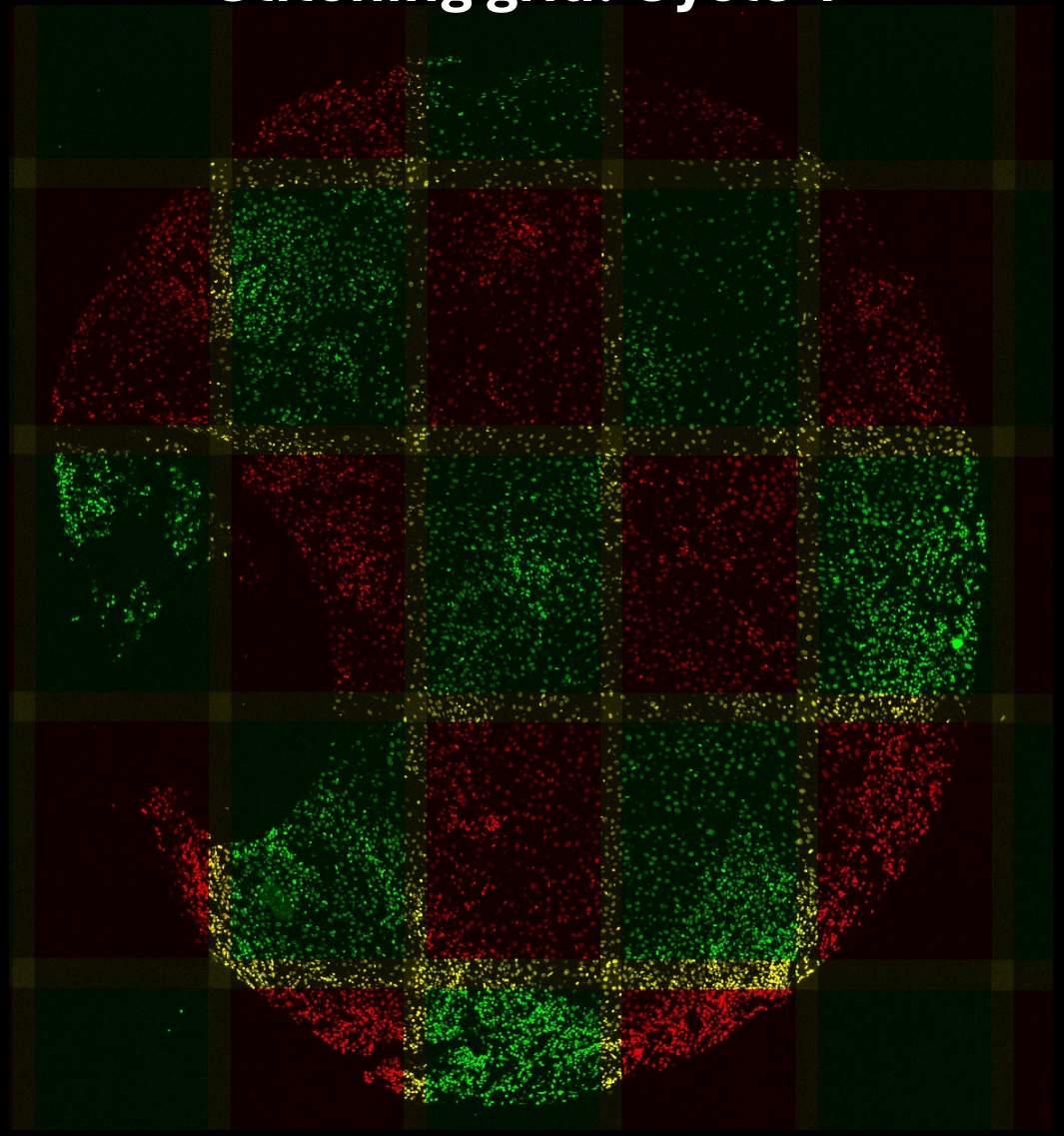


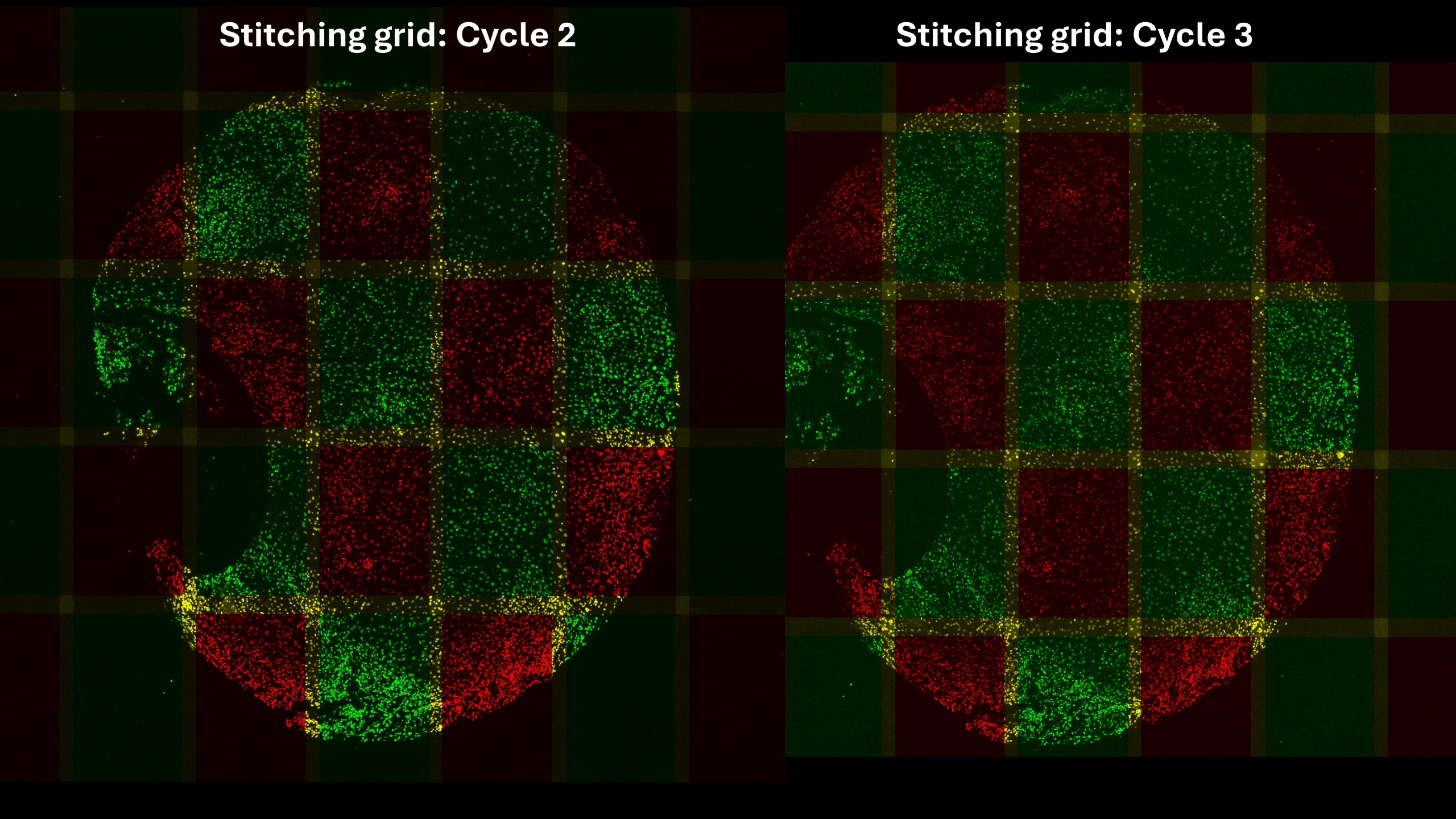
Blurryness from stitching or registration? Stitching grids seem perfect...

Stitching grid: Cycle 0



Stitching grid: Cycle 1




Stitching grid: Cycle 2
Stitching grid: Cycle 3

Results from Stitching QC:

- Changing stitching parameters significantly improves output stack
  - 7um shifts still present
- Stitching seems perfect when checking the tile overlap

Conclusion:

- After improving the stitching parameters,
  the problem lies in the registration,
  this should fix that 7um shifts

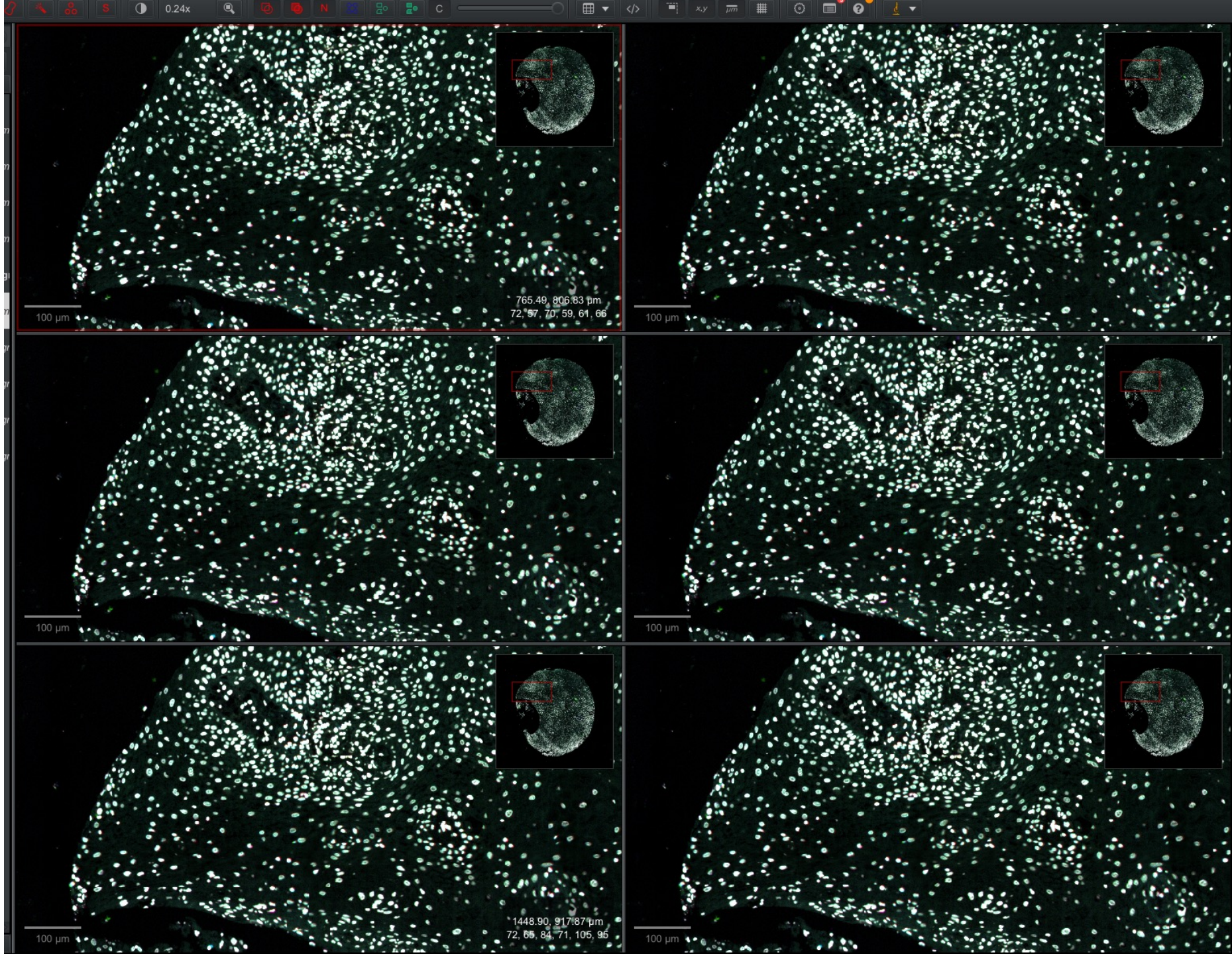
Question:
Passing shift and sigma values to registration process help?

Results:
Blurryness is gone, stitching and registration seem perfect.

Conclusion:
Registration was causing the issue, not stitching.

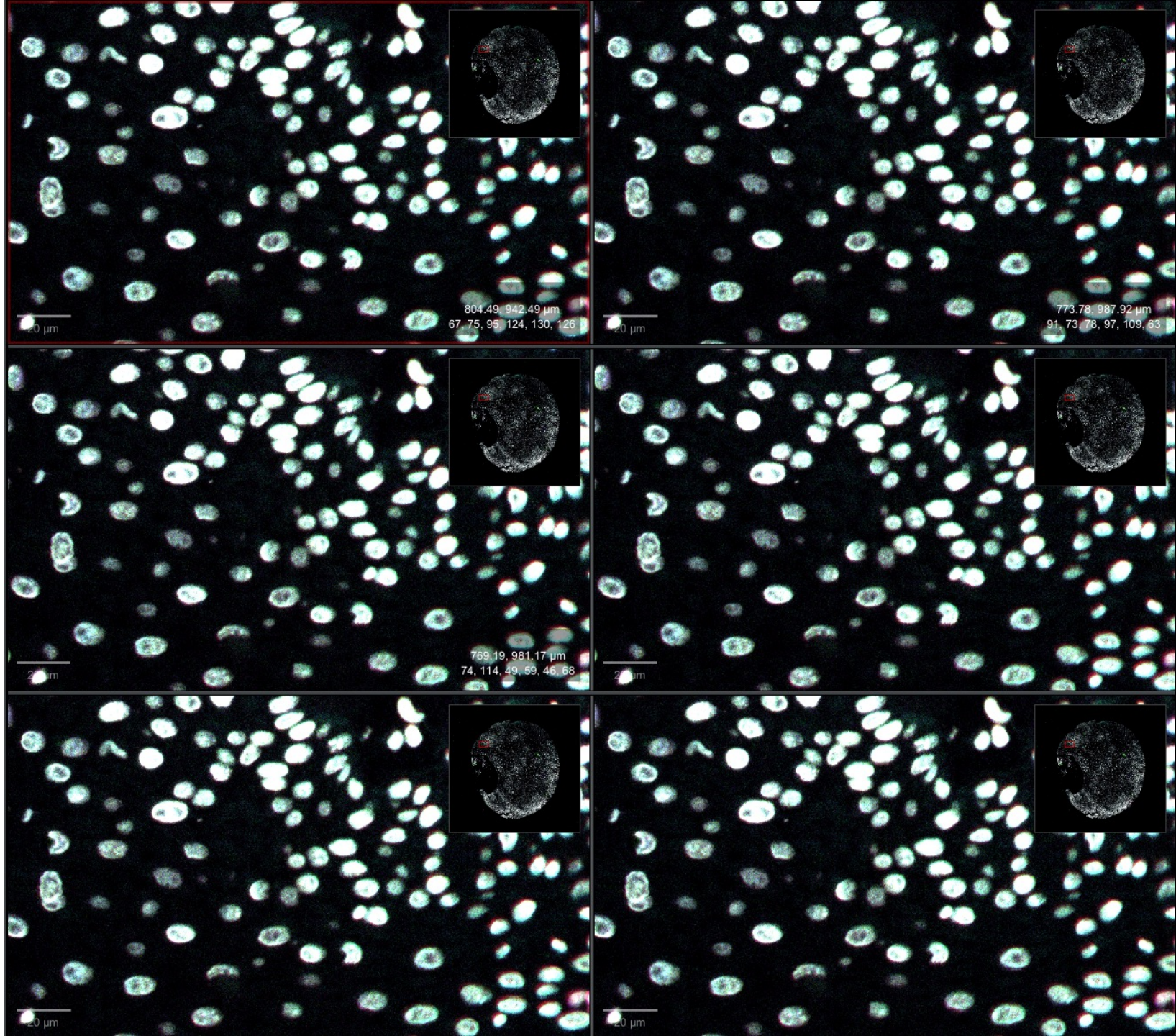
Shift 50 Sigma 1	Shift 50 Sigma 2
Shift 100 Sigma 1	Shift 100 Sigma 2
Shift 150 Sigma 1	Shift 150 Sigma 2

Also: Increasing shift greatly does not cause artefacts



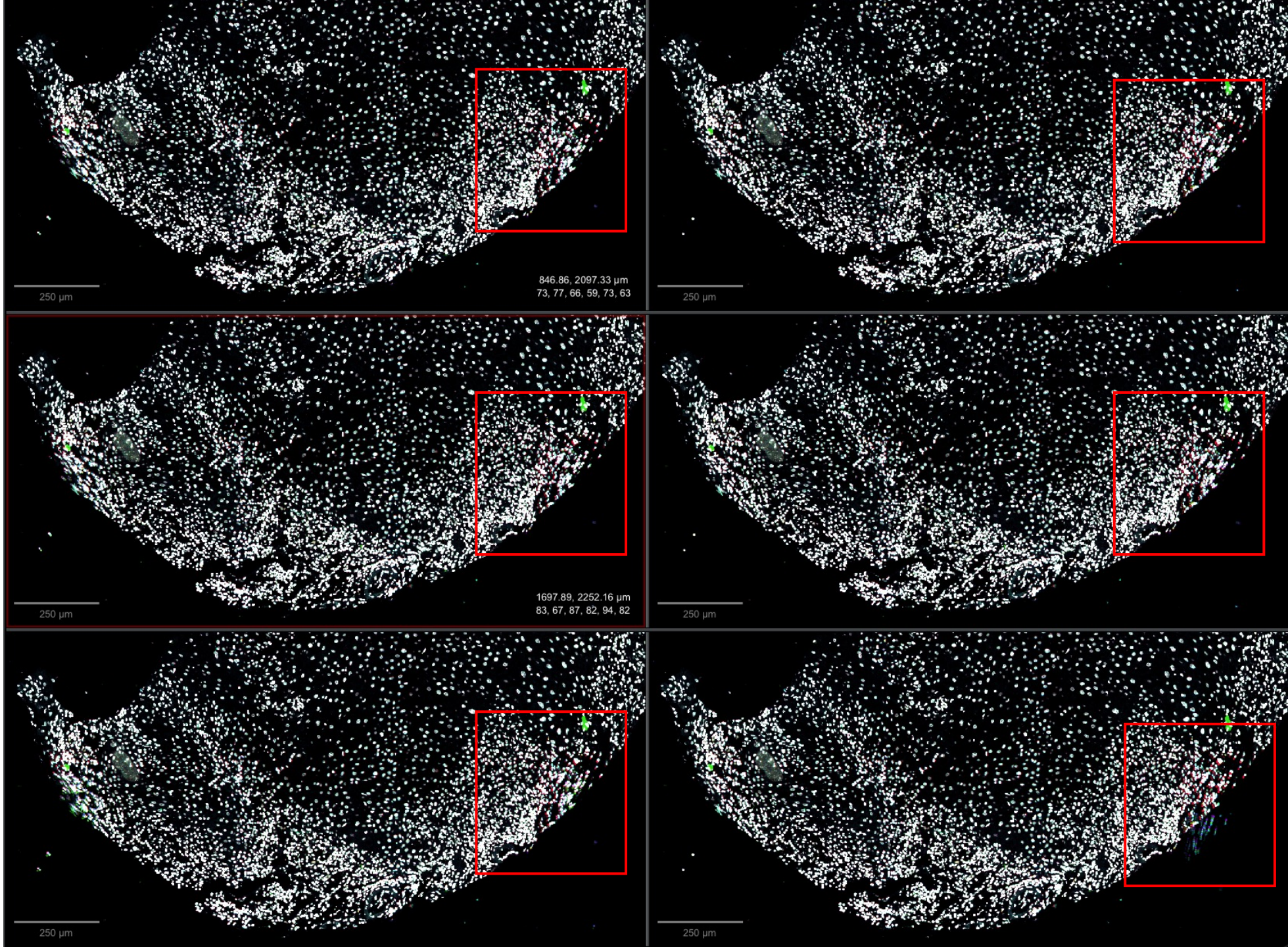
Same but closer

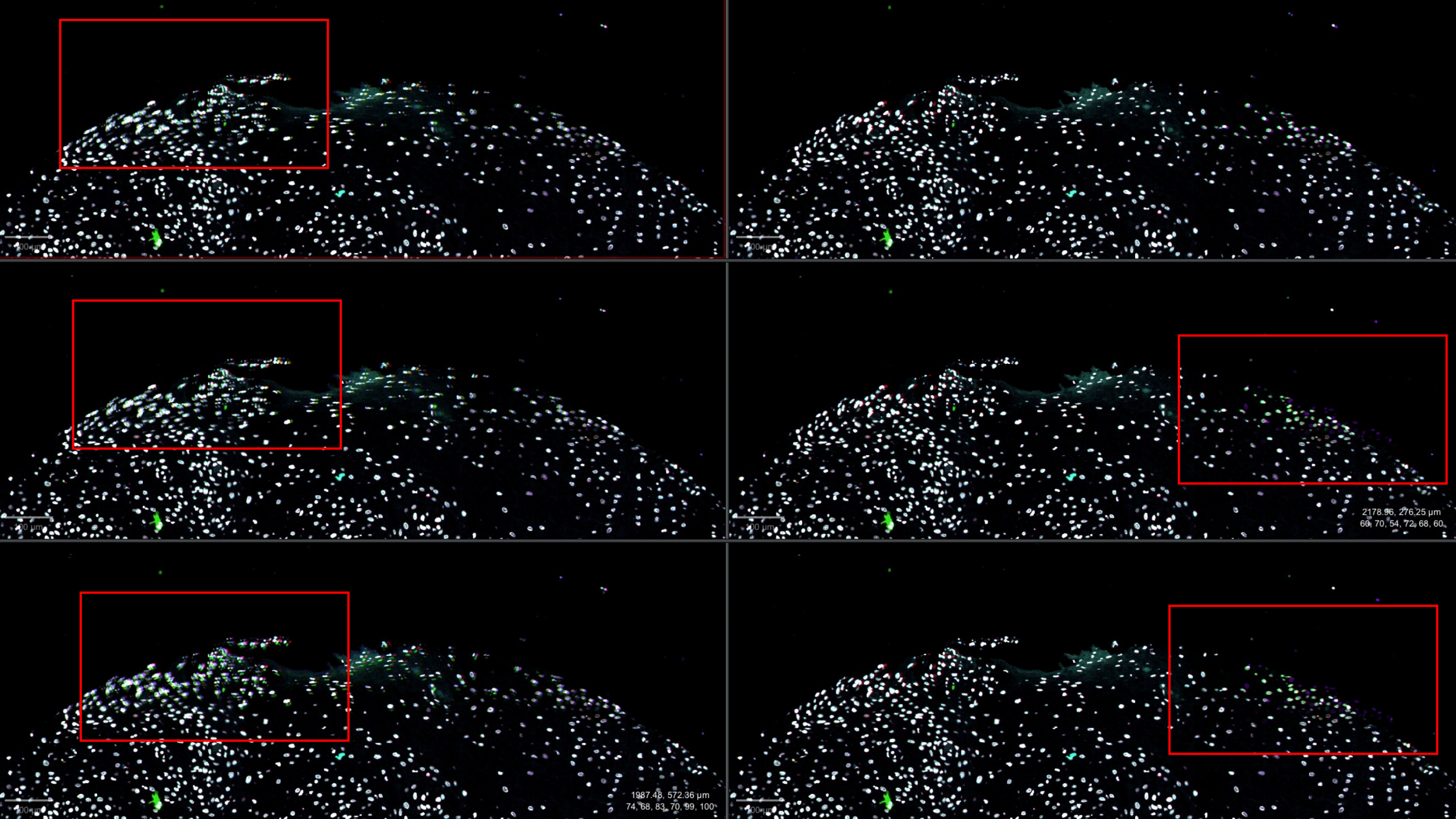
Shift 50 Sigma 1	Shift 50 Sigma 2
Shift 100 Sigma 1	Shift 100 Sigma 2
Shift 150 Sigma 1	Shift 150 Sigma 2



Still small issues with specific tiles

Shift 50 Sigma 1	Shift 50 Sigma 2
Shift 100 Sigma 1	Shift 100 Sigma 2
Shift 150 Sigma 1	Shift 150 Sigma 2




100 µm
100 µm
100 µm
100 µm
2178.96, 276.25 µm
60, 70, 54, 72, 68, 60
100 µm
1987.48, 572.36 µm
74, 68, 83, 70, 99, 100
100 µm
100 µm

Why are some tiles misbehaving?

I don’t know, but I have a hypothesis:

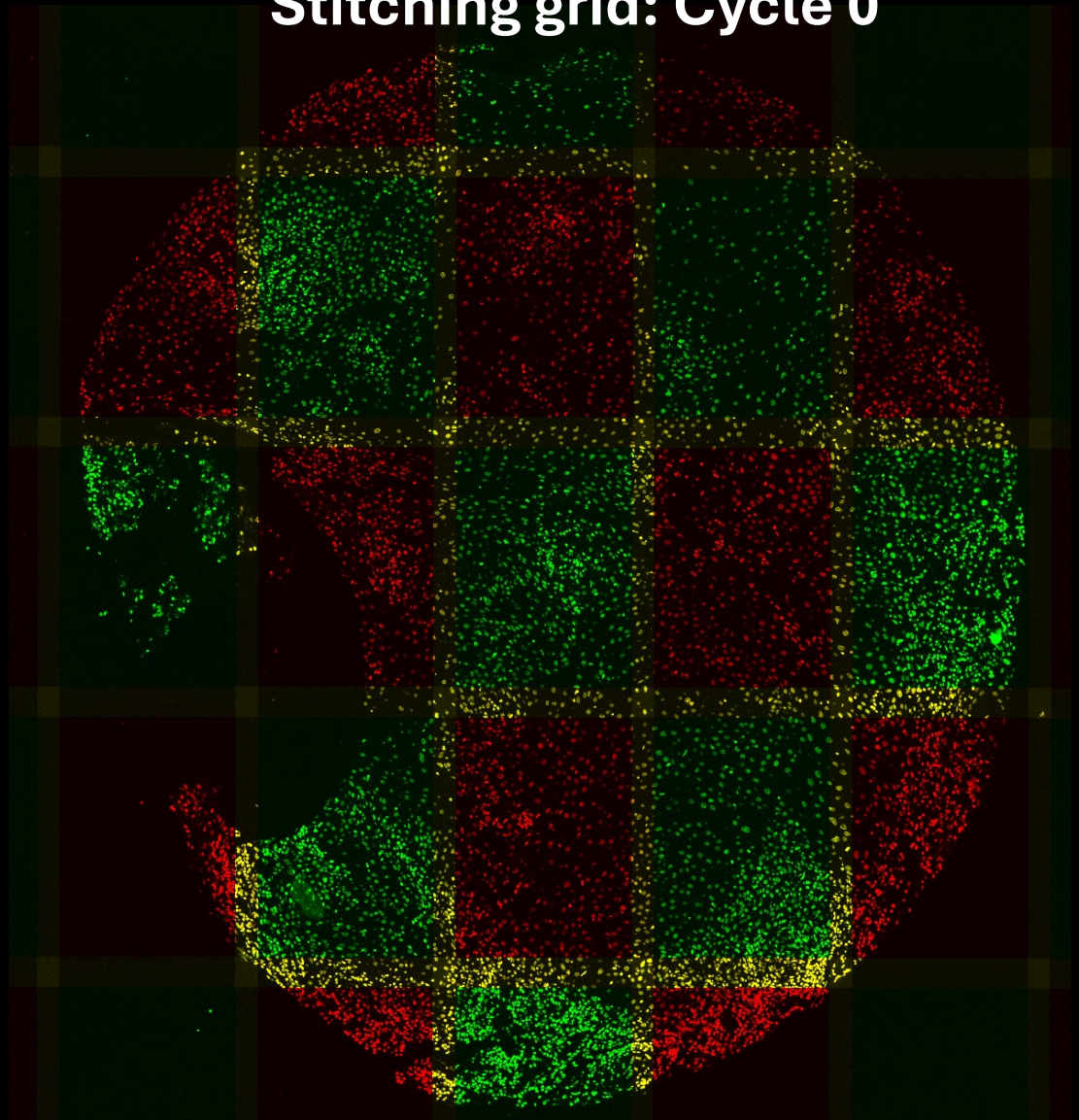
Tissue positioning on tiles changes through cycles, and sometimes there are large discrepancies that the math can’t deal with.

What do I mean with discrepancies?

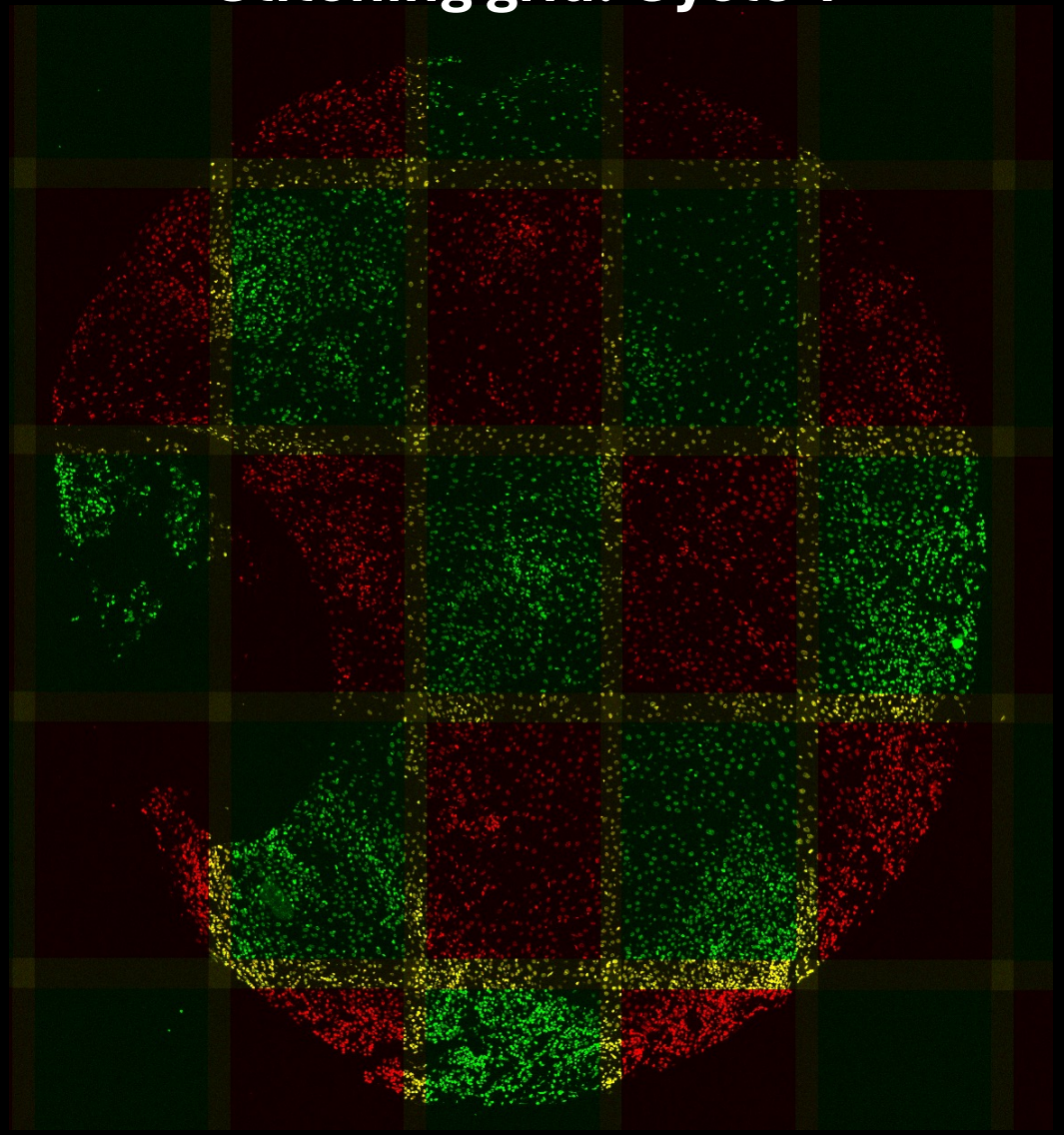
Look at this images again:

Look at the top tiles and the bottom right tiles

Stitching grid: Cycle 0

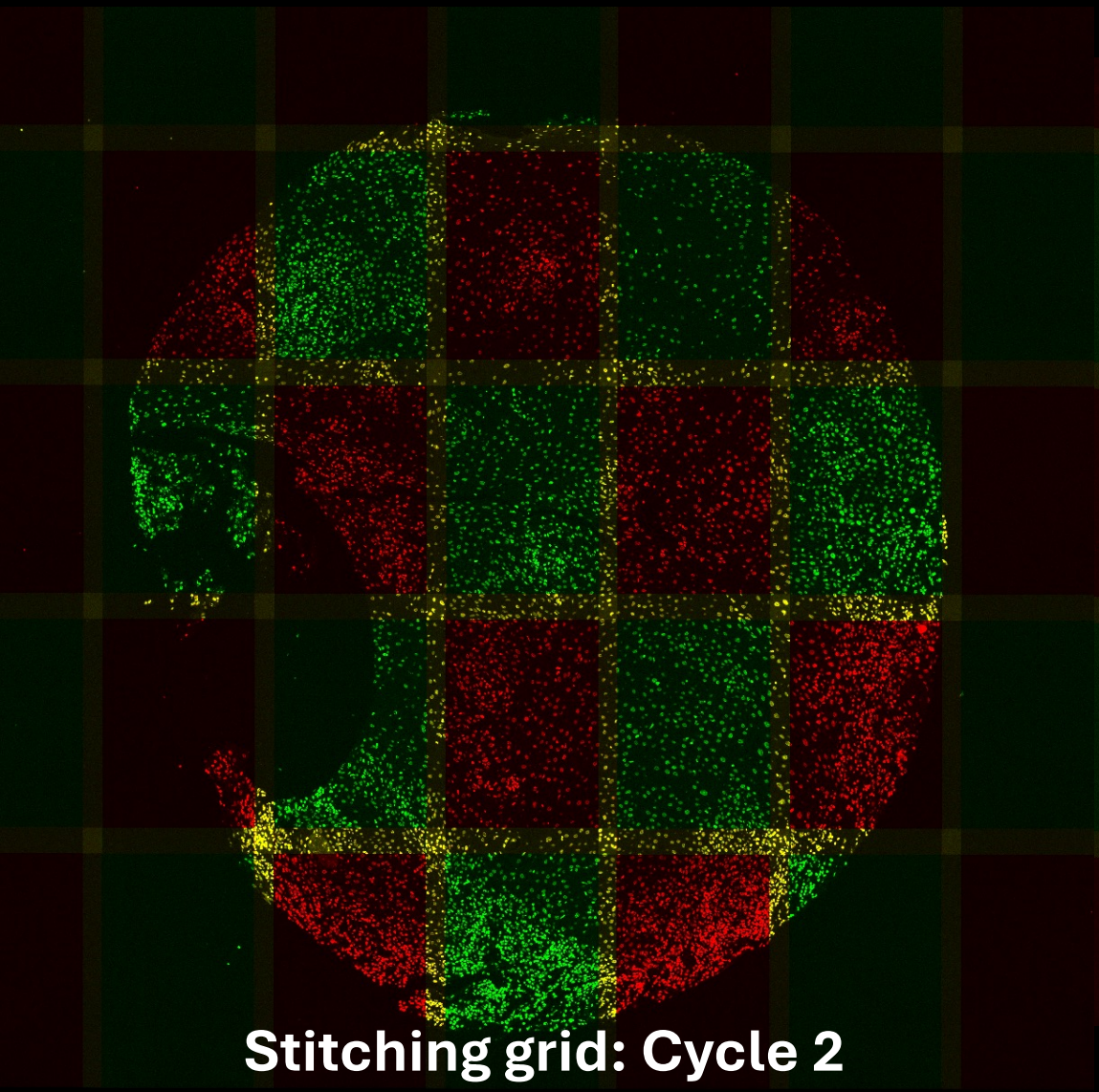


Stitching grid: Cycle 1

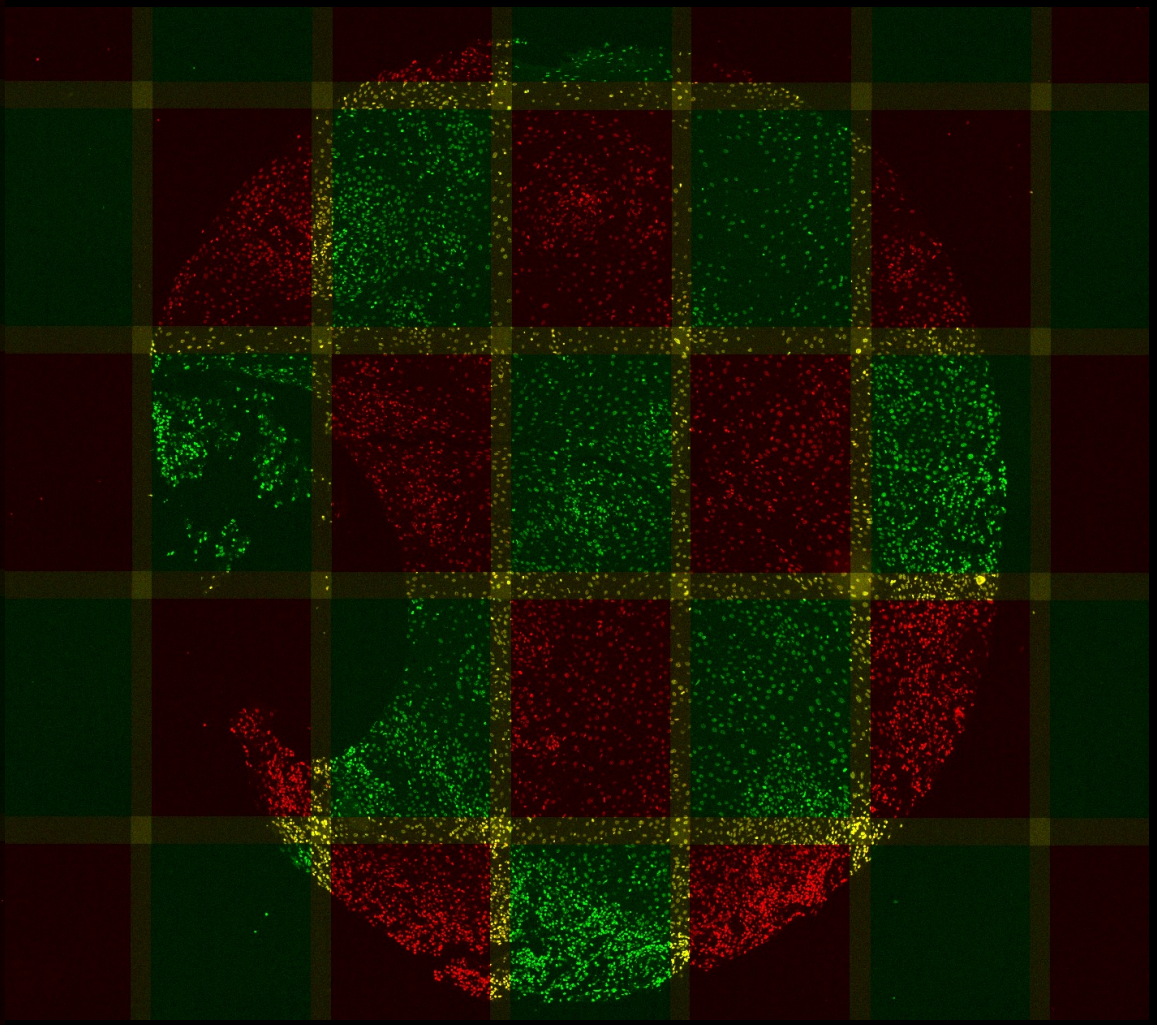


Look again.

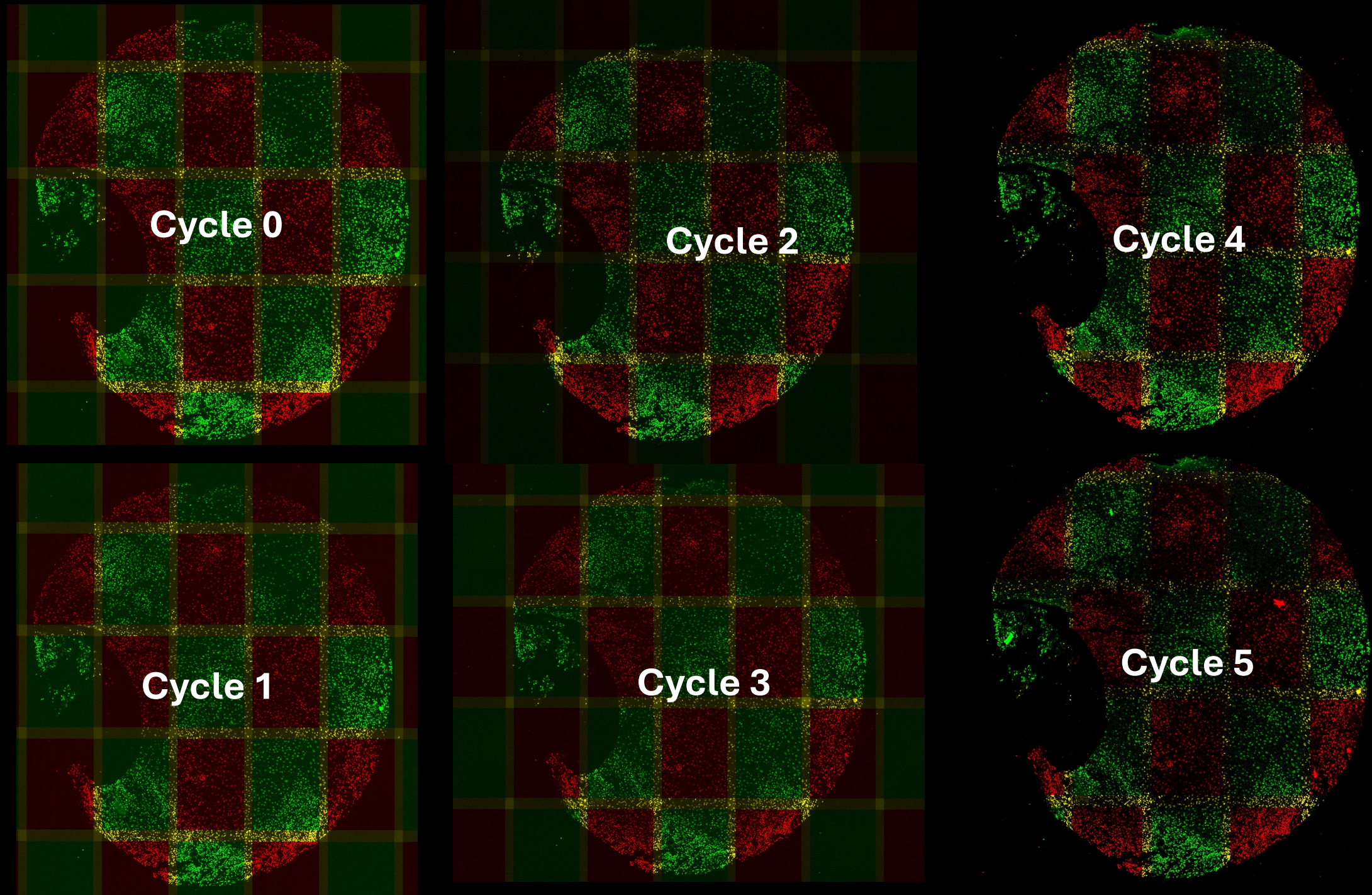
See how there is much less tissue on those tiles compared to before

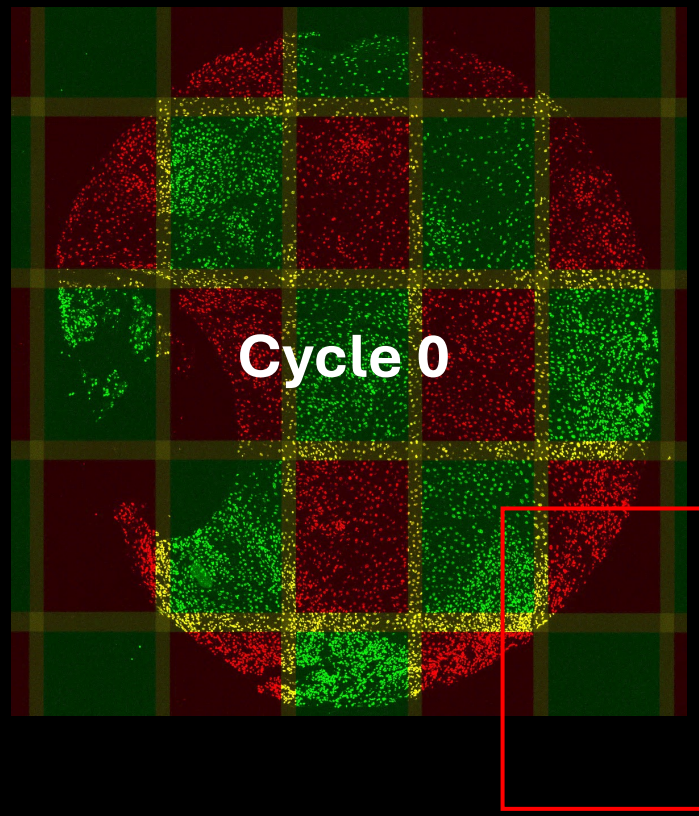


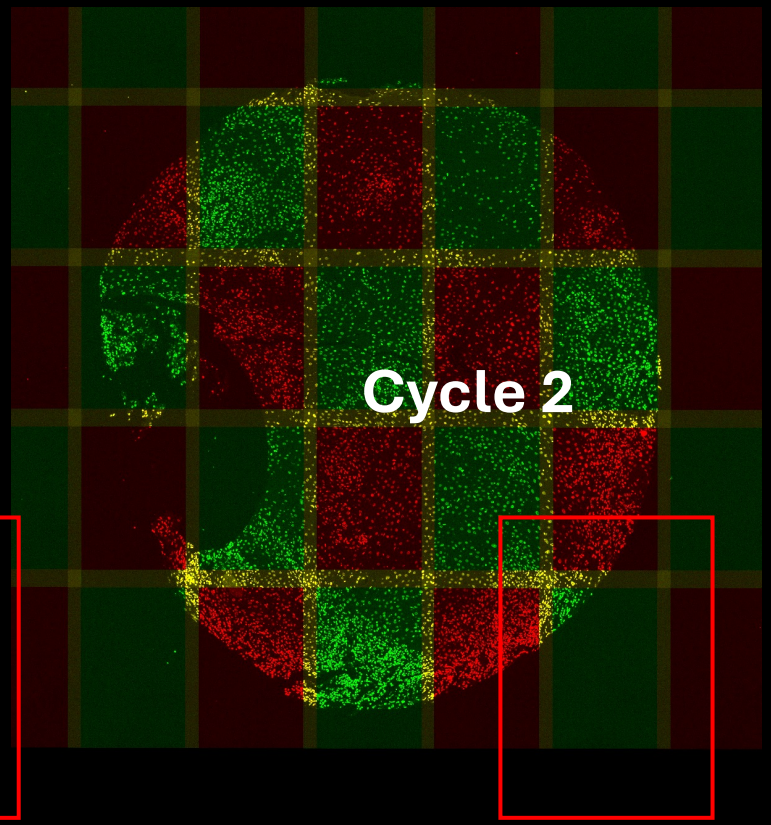
Stitching grid: Cycle 2

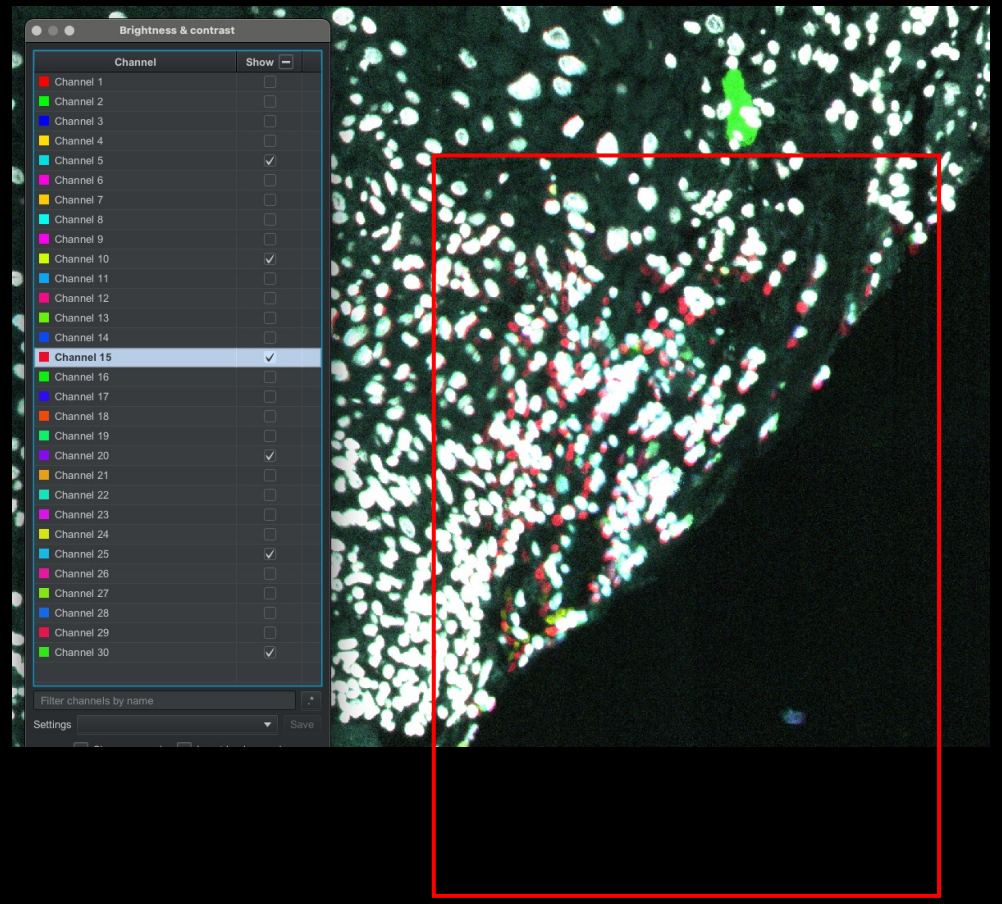


Stitching grid: Cycle 3

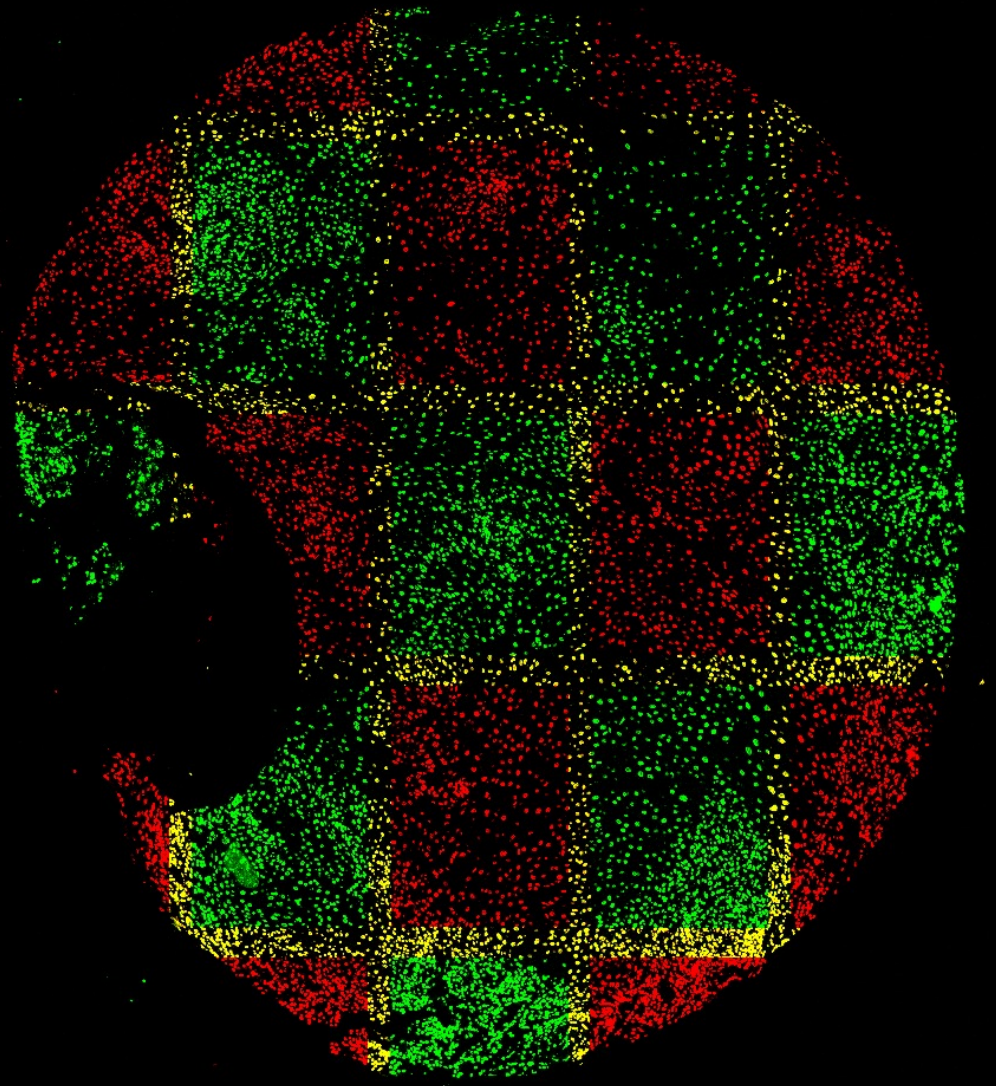

Cycle 0
Cycle 2
Cycle 4
Cycle 1
Cycle 3
Cycle 5

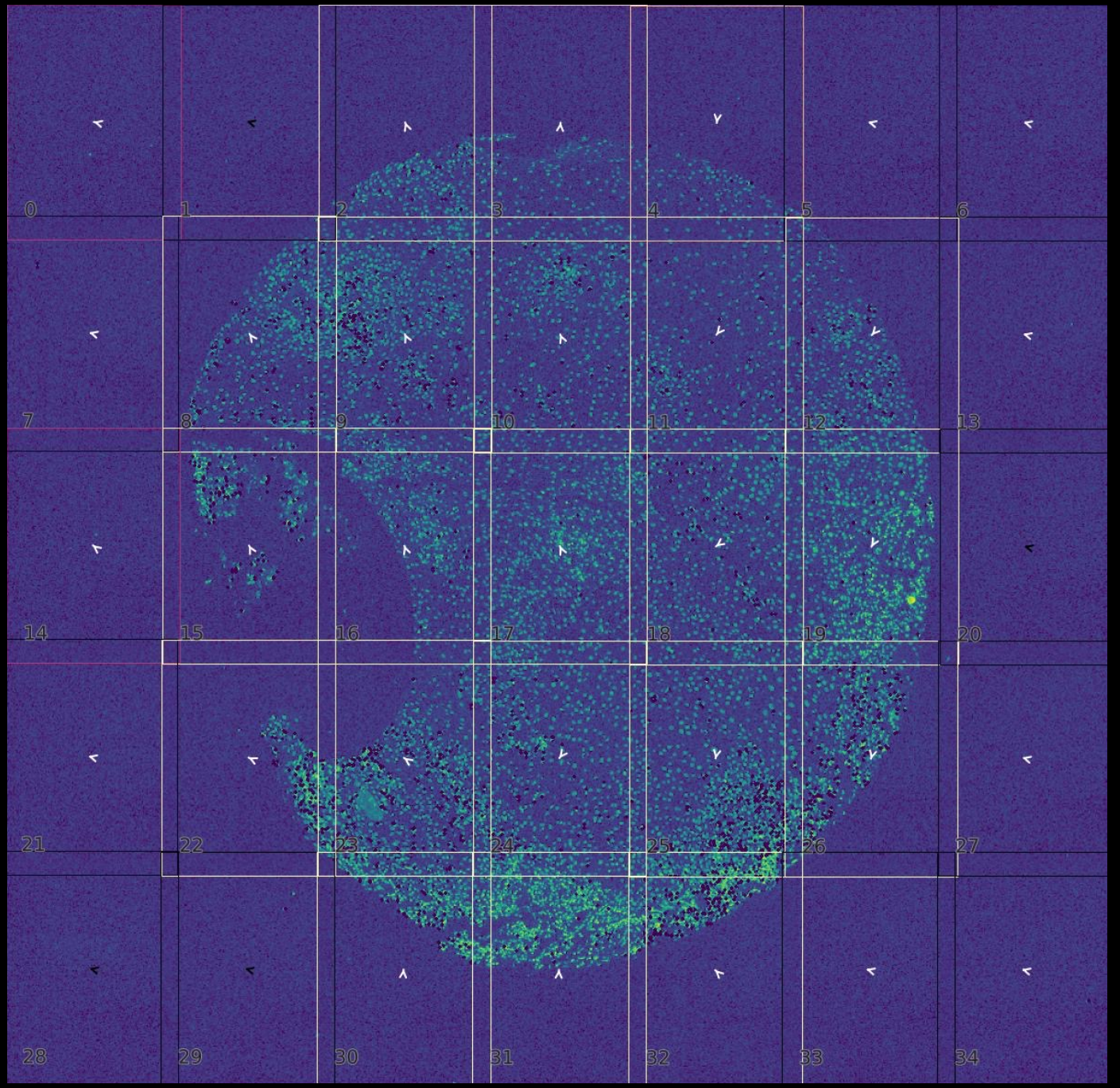






Hypothesis:
This difference is causing a mistake in registration




0
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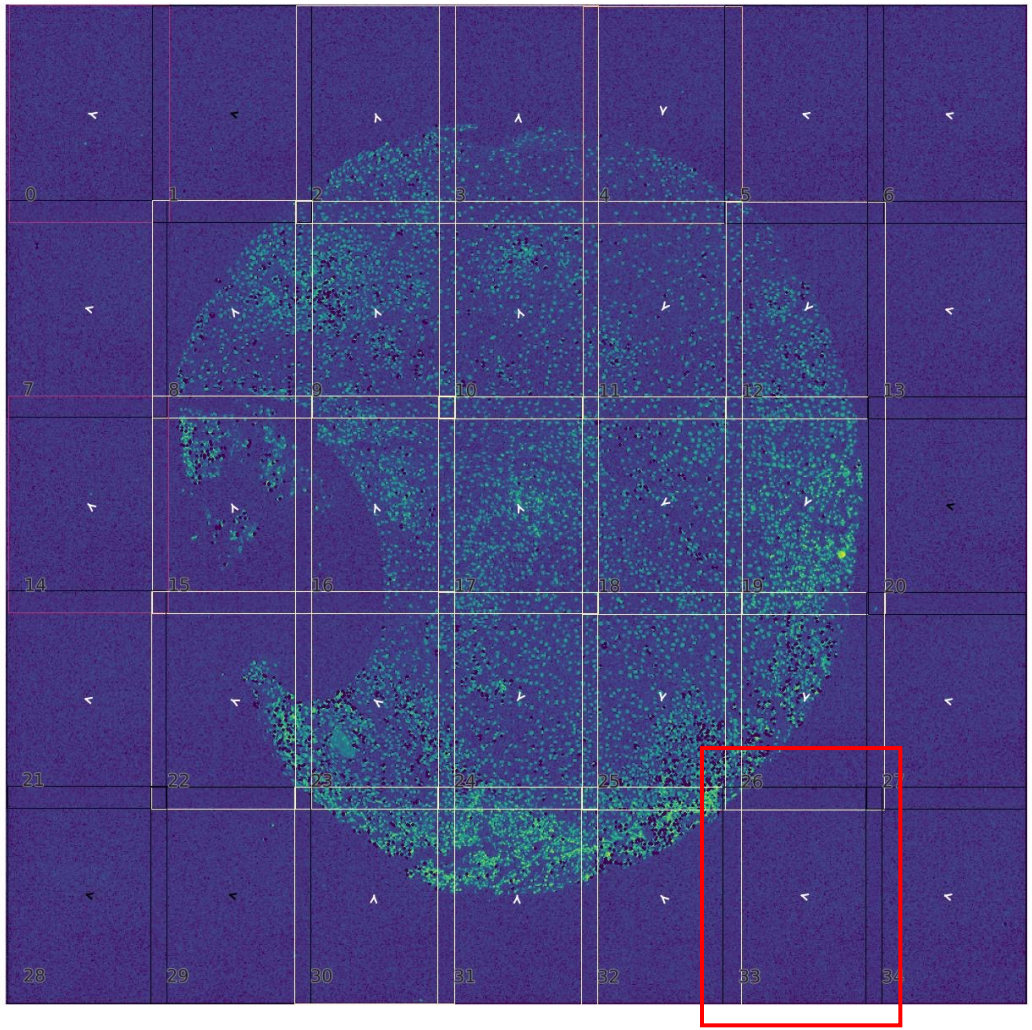
33
34

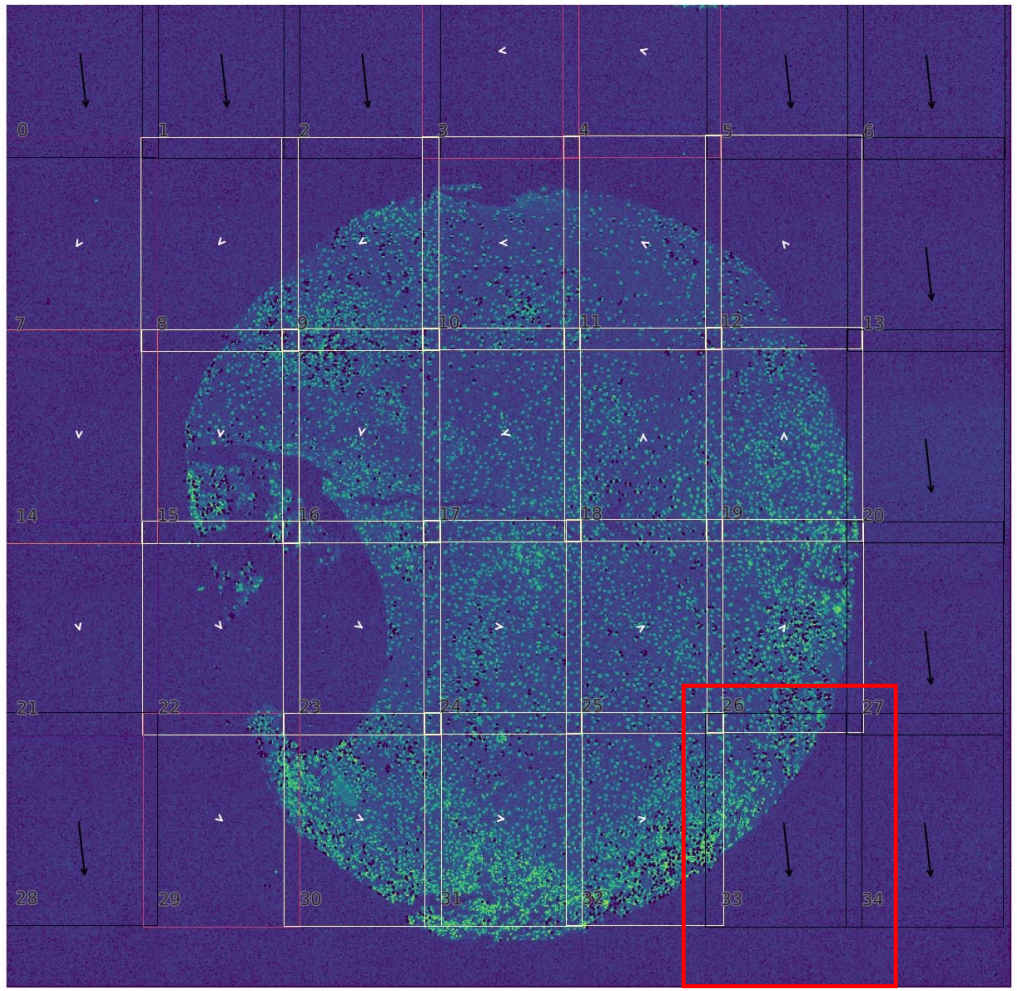
I double checked by measuring the registration shift per cycle.
Remember this is always done again a single cycle as reference, here cycle 0.

Black arrows mean too much error and will not register

Cycle 0-1



Cycle 0-2



Conclusion

- Increasing sigma and max_shift parameters significantly improves the stitching and registration.
- Small shifts still exists in specific tiles, most likely due to slide shifts between cycles, which leads to inconsistent tissue amounts for the same tile.

Next steps:

- Stitch and register whole TMA slides with new parameters (hopefully it scales up well)
- Allowing much better segmentation, quantification, and analysis.