2020 CYSF / YSC Online Date

STEM Fair

Past Project

Summary:

30 ug/L atrazine

30 ug/L "

1000 ug/L "

2000 ug/L "

Tested effects on daphnia magna

Results

1. increased expression of 32 kPa protein
2. decreased protein ubiquitination
3. 3 and 30 ug/L - no effect on F1

1000 and 2000 ug/L - decrease ~~number of~~ F1

2021 CYSF

Question: Can ~~Apoptosis~~ ~~Ubiquitination~~ be linked to decrease in number of F1

Date

Prepare atrazine stock solution: 1mg/ml

1. weigh 3mg atrazine
2. add 3ml EtOH (100%) - cover immediately
3. mix well - make sure atrazine is dissolved completely

* make sure to "zero" the scale after putting the weighing boat

4. aliquot into eppendorf tubes:
   400ul/tube - about 7ml total (will lose some during aliquoting)
   put parafilm around lids

dechlorinated water
4L - 200ml
2L - 100ml
1L - 50ml
50ml 25ml

Prepare 40ml of 1000ug/L and 2000ug/L

Stock 25% solution 1×10^6 ug/L

$\frac{(40 \times 1000)}{(1 \times 10^6)} = 40ml$

$\frac{(40)(2000)}{1 \times 10^6 ug/L} = 80ml$

Day = # of days in advance

(paraformaldehyde)

Day	treatment	tube	Frozen			PFA Date	
7	Cx - 1000	13	10				5
	A - 1000	14	10	5			3
	Cx - 2000	15	9				3
	A - 2000	16	10				6
4	Cx - 1	9	10	6			3
	A - 1	10	11	10	* 7		3
	Cx - 2	11	10		Testing		9 total
	A - 2	12	9				3
2	Cx - 1	5	12	11			3
	A - 1	6	[illegible]	11			3
	Cx - 2	7	11				3
	A - 2	8	12	11			5
1	Cx - 1	1	12				3
	A - 1	2	12				3
	Cx - 2	3	12	3			3
	A - 2	4	12	3			3

F1 (all d4)

		Frozen		PFA
17	Cx - 1	[illegible]	6	5
18	A - 1	10		7
19	Cx - 2	[illegible]	6	5
20	A - 2	10		3

Apoptosis assay: DNA fragmentation assay

Reagents:
- Agarose
- DNA ladder
- GelRed (protect from light exposure)
- TAE (Tris-acetate-EDTA) buffer

Prepare 1.5% agarose gel

1. Measure amount of agarose needed (can try 1% or 1.2% in practice run)
2. Mix agarose with 1X TAE in a microwavable flask (TAE Recipe)
3. Microwave in pulses (~30 sec; swirling the flask occasionally as the solution heats up) until the agarose is completely dissolved (1 to 3 min; do not overboil as some of the buffer will evaporate and alter the final percentage of agarose in the gel.
   CAUTION: *HOT! Be careful stirring, eruptive boiling can occur.*
4. Let agarose solution cool down (about 5 min) to about 50 °C (when you can comfortably keep your hand on the flask)
5. Add SYBR Safe DNA stain to the solution at 1:10,000 dilution
6. Pour the solution into a gel tray with the well comb in place.
   - Pour slowly to avoid bubbles. Bubbles can be pushed away from the well comb or towards the edges of the gel with a pipette tip.
7. Let the solution sit at room temperature for 20-30 mins (or at 4 °C for 10-15 min) until it has completely solidified
   - If you are in a hurry, the gel will set more quickly if gel tray is placed at 4 °C earlier so that it is already cold when the gel is poured into it

Agarose gel electrophoresis

8. Once gel is solidified, remove the gel combs; gel must be in tank with 1X TAE buffer.
9. Load DNA ladder and DNA samples (500 ng, 1 ug or 2 ug)
10. Run agarose gel in electrophoresis apparatus at 125 volts for 45 min (large gel)
11. Visualize gel in BioRad Gel imager/Blue light LED illuminator
12. Take photos

DNA islation: DMSO-SDS-TE method

Reagents:

DMSO (dimethyl sulfoxide)
PBS (Phosphate-buffered saline)
2% SDS-TE (Tris-EDTA) buffer, (pH 7.4); (dissolve 0.1g SDS in 5 ml TE)

- Wear a mask when weighing SDS (sodium dodecyl sulfate)

1. Prepare 7 (at least 3) Daphnia - wash with PBS 1X- centrifuge (set to go up to 10,000 rpm) , 5 sec
2. Discard supernatant
3. Add 100 ul (50 ul if 3 Daphnia) DMSO to Daphnia pellet
4. Mix well and immediately vortex (4 x 10 sec)
5. Add equal volume (100 ul; 50 ul if 3 Daphnia) of 2% SDS-TE
6. Mix and vortex (4 x 10 sec)
7. Centrifuge at 12,000 g at 4°C for 5 min
8. Transfer supernatant (with isolated DNA) into new eppendorf tube
9. Measure DNA concentration and purity
    - UV-spectroscopy: record absorbance at 260 (concentration) and 280 nm
    - "**A260** unit" is the quantity measure for nucleic acids.
    - 1 **A260** unit = amount of nucleic acid in 1 ml, producing OD = 1
    - 1 **A260** unit dsDNA = 50 μg.
    - 1 $\mathbf{OD}_{260}$ (optical density) = amount of DNA that gives an absorbance reading = 1.0 at a wavelength of 260 nm for a sample in 1.0 ml ddH_2O, read in a 1 cm quartz cuvette.
    - Calculate A260/A280 to analyze purity; good-quality: A_{260}/A_{280} ratio= 1.7–2.0

Collected Samples

Date

Tube

		# of dys in atrazine	
1	Cx - 1	day 1	Treated with atrazine at day 7
2	A - 1		
3	Cx - 2		
4	A - 2		
5	Cx - 1	day 2	
6	A - 1		
7	Cx - 2		
8	A - 2		
9	Cx - 1	day 4	
10	A - 1		
11	Cx - 2		
12	A - 2		
13	Cx - 1	day 7	
14	A - 1		
15	Cx 2		
16	A - 2		
Fl			
17	Cx - 1	day 4	Burn in ± atrazine
18	A - 1		
19	Cx - 2		
20	A - 2		

3 dyphnias ~~for~~ each treatment for DNA ladder assay and microscopy

Page

Prepare buffers

TAE - Tris acetate EDTA
TE - Tris EDTA
PBS - Phosphate buffered saline
PBST - PBS with 0.1 % tween - 20
2 % SDS in TE buffer
0.1% Triton X 100 in PBS

$C_i V_i = C_f V_f$ TE buffer

10 mM Tris pH 7.4 (pH 8.0)
1 mM EDTA

stock conc	Final
50 mM Tris pH(7.4)	10 mM
500 mM EDTA pH (7.6)	1 mM

1 ml 1 M Tris (pH 8.0) 10 mM
0.2 ml 0.5 M EDTA (pH 8.0) 1 mM

100 ml

TE buffer with 2% SDS

Date

20 ml

$\frac{1}{50} \times 20\,ml = \frac{2}{50}\,ml = 0.04\,ml$

$= 40\,\mu l$

$\frac{20\,ml \times 10\,mM}{50} = \frac{200}{50}\,ml = 4\,ml$

4 ml of 50mM Tris pH 7.4

40 µl of 0.5M EDTA

4.04 ml

15.96 ml of d.d w = 20 ml

If made 40 ml

40 ml × 0.02 = 0.8 g

4 + 4 ml of 50mM Tris pH 7.4

40 + 40 µl of 0.5 EDTA

DAPI Lec 365 &180 9192

Lec 314 74-99

Lec 336A 99

1× PBS

7.5 µl + 142.5

= 150 µl

0.5 µg/ml DAPI 150 µl

$\frac{0.5\,\mu g/ml \times 150\,\mu l}{10\,\mu g/ml} = \frac{75}{10} = 7.5\,\mu l$

Date

1 % Agarose 180ml

180 ml 1 % agarose

DNA dye: redsafe 20 000 x

180 x 0.01 = 1.8g agarose in 180ml TAE buffer

720ml of TAE ~~buffer~~

use 50x TAE

$\frac{720}{50} = 14.4ml$

720 - 14.4 = 706ml of d.dW

For final expt prepare minigel 1.2 % agarose

50ml x 0.012 = 0.6g

add redsafe dye to 1x

$\frac{50ml}{20\,000} = 2.5\mu l$

Samples

Date

Frozen	D1	D2	D4	D7
C - 1	12	8	12	12
A - 1	12	10	12	14
C - 2	12	9	12	13
A - 2	12	10	12	14
C - F1	8	10		
A - F 1		11		

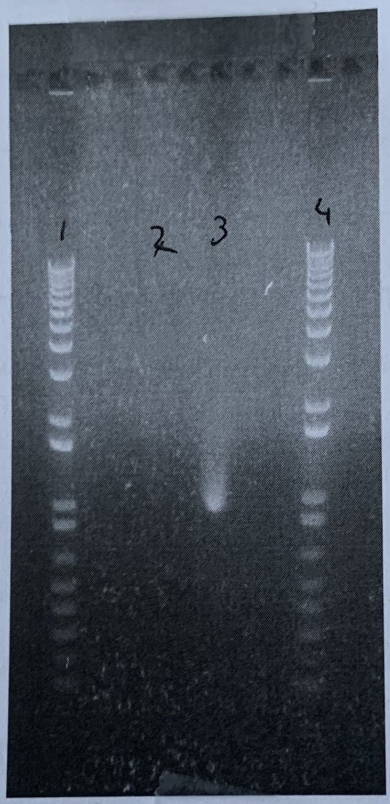
DFA

C - 1	12	11	12	15
A - 1	12	12	12	16
C - 2	12	12	12	16
A - 2	12	14	12	18
C - F1		12		
A - F1		11		

Practice run

Date

Trial DNA ladder Assay



DNA concentration

Sample 31.2 ng/ul = A260 nm

purity: A260/280

= 1.76

A260/A230

= 0.32

Voltage 125

Time: 40 min

1-2 % gel

1 DNA ladder

2 800 ng DNA sample

3 1 ug

4 DNA ladder

Next time use at least 1 ug

Date

31.2 ng / ul DNA × 400 ul

total V = 12.48 ug

in 7 daphnia

$\frac{12.8 \text{ ug}}{7} \simeq 1.7 \text{ ug}$ DNA / daphnia

Prepare at least 3 daphnia to get 5.1 ug

Date

Measure DNA concentration, purity / quality (A260/A280)
and contamination (A260/A230)

Abs 260 - DNA

Abs 280 - protein

Abs 230 - contamination

Nucleic acid maximum absorbance at 260nm

A260/280 ratio of ~1.8 - pure DNA

(ratio ~2 - pure RNA)

Low A260/A230 - due to contamination
with EDTA used in purification method
↓
DNA

ORDER OF SAMPLES IN GEL

Date

						H_2O
	1	empty	10× dye Sol			
	2	ladder				
D1	3	sample 1	MM 4	0.0414ug/ul	24.15ul	7.85
	4	2		0.0224	44.64ul	
D2	5	5		0.0289	34.60	
	6	6		0.0351	28.49	3.51
1000ng/L D4	7	9		0.0439	22.78	8.22
atazir	8	10		0.0306	32.67	
D7	9	13		0.0371	26.95	4.05
	10	14		0.0623	16.05	15.95
F1	11	17		0.1551	6.447	25.553
	12	18		0.0439	22.77	8.23
	13	ladder	5			
D1	14	sample 3	MM 4	0.0233	42.92	
	15	4		0.0225	44.44	
D2	16	7		0.0548	18.25	13.21
	17	8		0.0320	31.25	0.25
2000ng/L D4	18	11		0.0444	22.52	8.48
atazir	19	12		0.0605	16.53	14.47
D7	20	15		0.0838	11.93	19.57
	21	16		0.0363	27.55	3.45
F1	22	19		0.0966	10.35	24.65
	23	20		0.0606	16.50	15.00

H_2O 0.1
approx 11.85

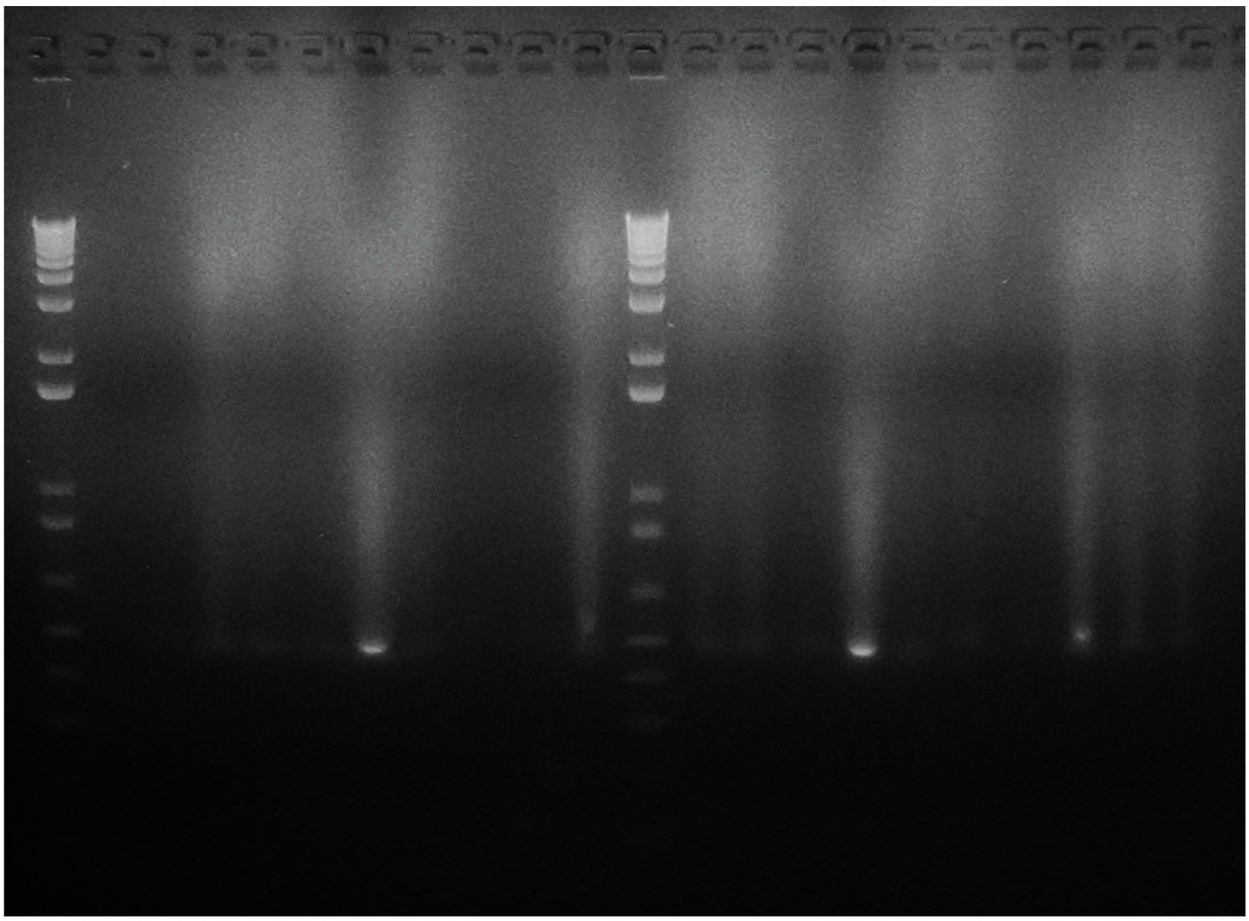
1.4
7.51
13.22
3.33
6.65
19.95
29.553
13.23

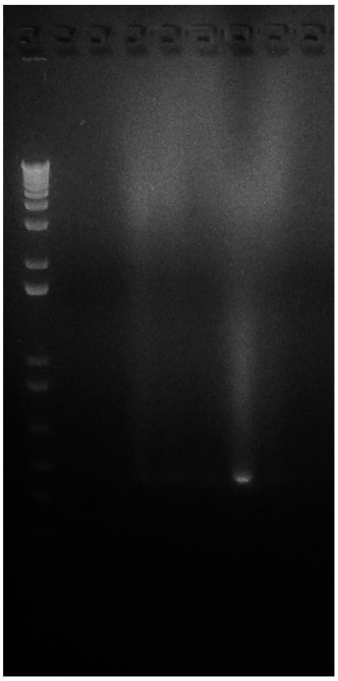
17.75
4.75
13.458
19.417
24.07
8.415
25.65
19.5

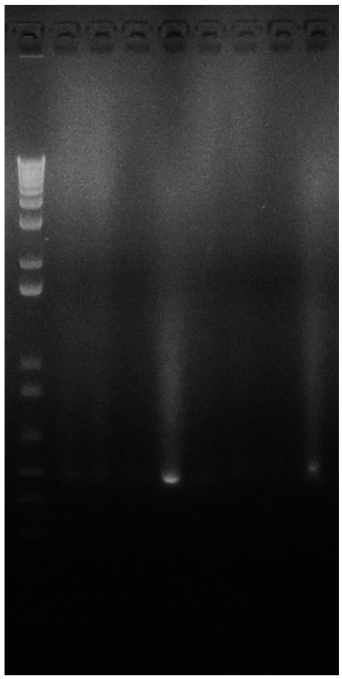
C]

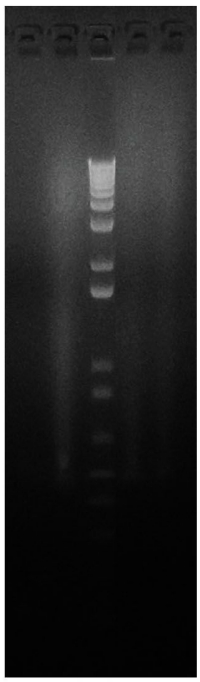
		A260/A280	A260/A230
1.	41.4 ng/ul	1.63	0.22
2.	22.4 ng/ul	1.68	0.20
3.	23.7 ng/ul	1.69	2.68
4.	22.5 ng/ul	1.71	0.98
5.	28.9 ng/ul	1.59	0.35
6.	35.1 ng/ul	1.88	-2.13
7.	54.8 ng/ul	1.81	1.31
8.	32.0 ng/ul	1.69	1.04
9.	43.9 ng/ul	1.92	-4.42
10.	30.6 ng/ul	1.86	-4.18
11.	44.4 ng/ul	1.68	0.81
12.	60.5 ng/ul	1.74	~~1.69~~ 0.45
13.	37.1 ng/ul	1.65	1.69
14.	62.3 ng/ul	1.78	-1.63
15.	83.8 ng/ul	1.62	1.53
16.	36.3 ng/ul	1.60	0.29
17.	155.1 ng/ul	1.66	2.29
18.	43.9 ng/ul	1.63	0.51
19.	96.6 ng/ul	1.66	0.60
20.	~~66.1 ng/ul~~	~~1.60~~	~~-34.25~~

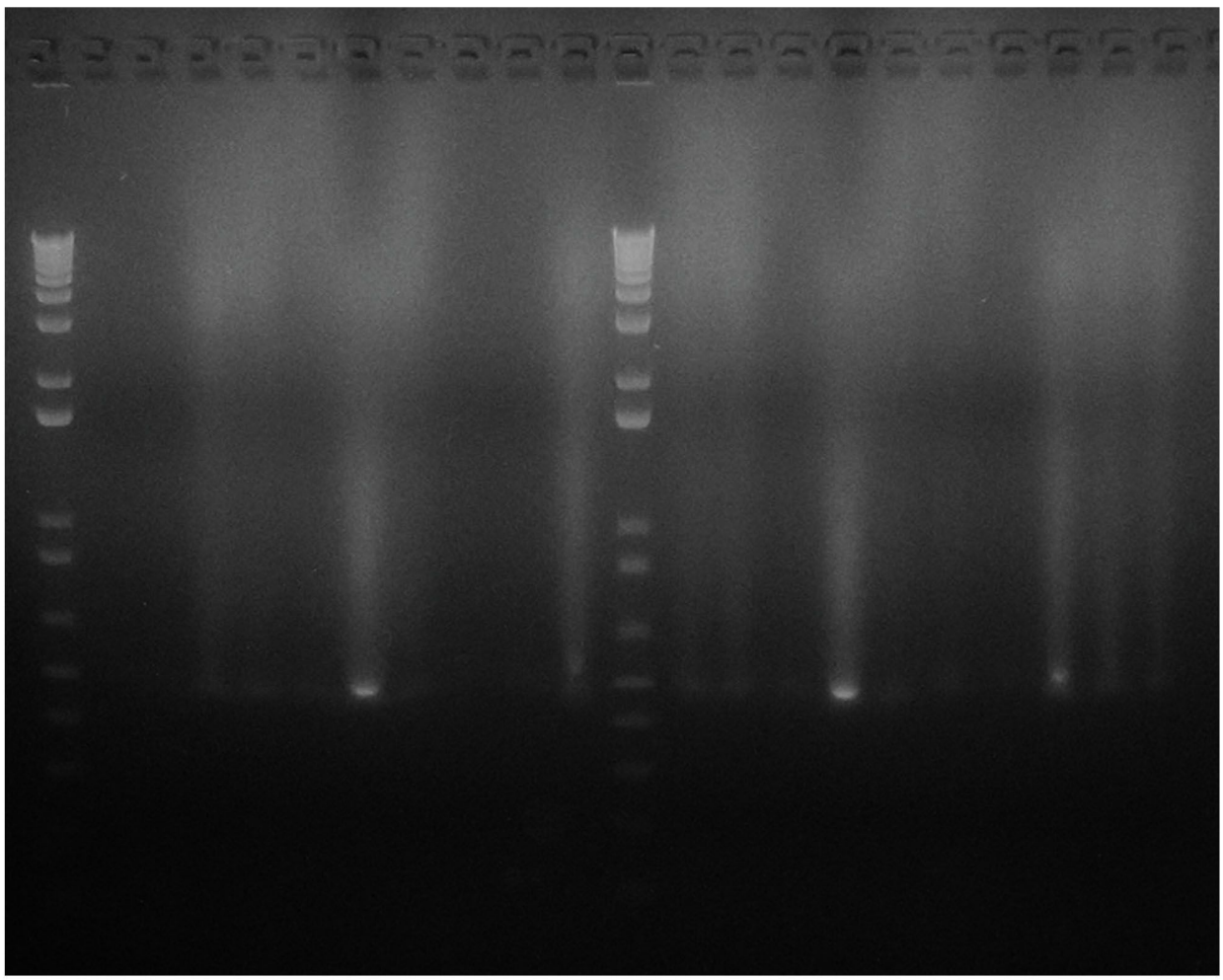
↳ 60.6 ng/ul 1.64 A260/A280 1.15 A260/A230

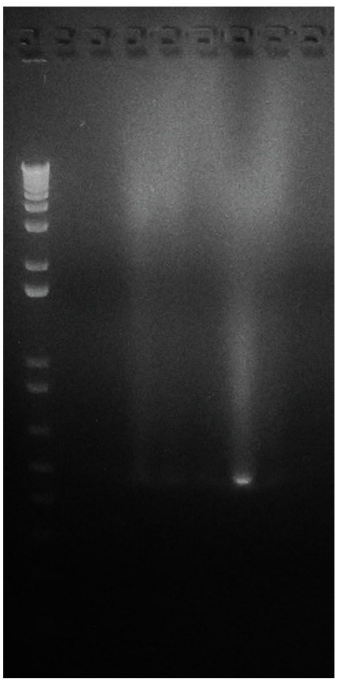


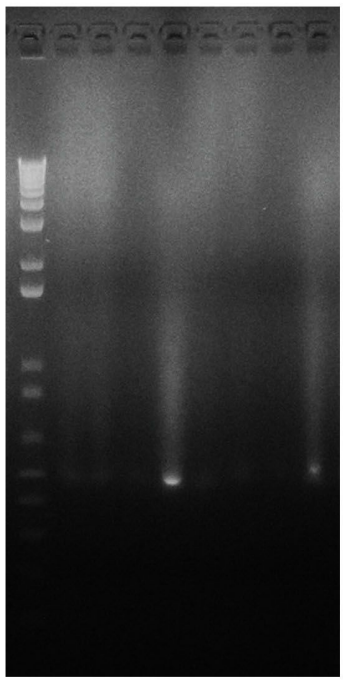


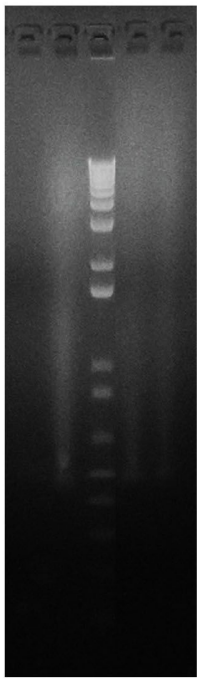












Immunofluorescence/fluorescence staining for activated caspase-3

Reagents:
PBS; used as medium throughout to preserve tissue structure
4% paraformaldehyde in PBS
0.1% Triton X-100 in PBS
Activated caspase-3 (1:50 in PBST)
DAPI (0.5 ug/ml)

1. Wash Daphnia in PBS 2X
2. Fix in 4% paraformaldehyde in PBS for 6 h
3. Wash in PBS 3X: 5 min, 60 min, 5 min
4. Permeabilize in 0.1% Triton X-100 in PBS overnight
5. Antigen retrieval: 20mM Tris-EDTA buffer pH 9.0; 95˚C; 10 minutes
6. Wash 2X in PBS; 5 min/wash
7. Stain for 48 hrs at 4˚C with: activated caspase-3 (1:50 in PBST); overnight with DAPI (0.5 ug/ml)
8. Wash 4X in PBS; 5 min, 10 min, 5 min, 5 min
9. Mount individual *Daphnia* in glycerol; seal coverslips with nail polish
10. Immunofluorescence/fluorescence microscopy; take photos

Photoshop instructions to bring back fluorescence color

Image
To color:
Mode- 8 bit
Mode- Indexed color
Mode- Color table- highlight all boxes that can be *highlighted
New box appears;

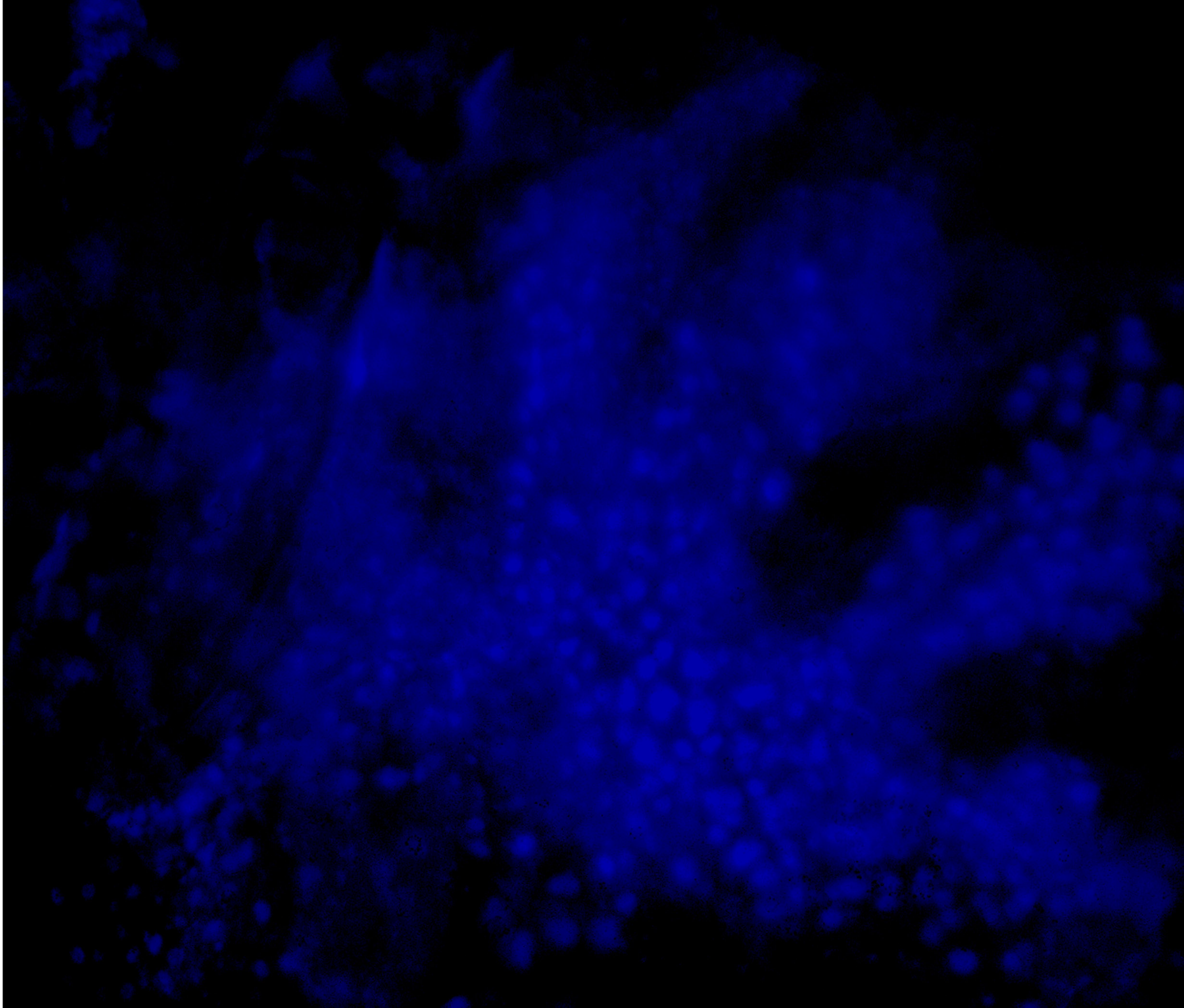
For dapi/blue: * [black first] enter 6 zeros in # box; click ok; while on the same screen:
[blue second] enter 2408f7, then ok for second screen and another ok for first screen
*if this doesn't work, do blue first

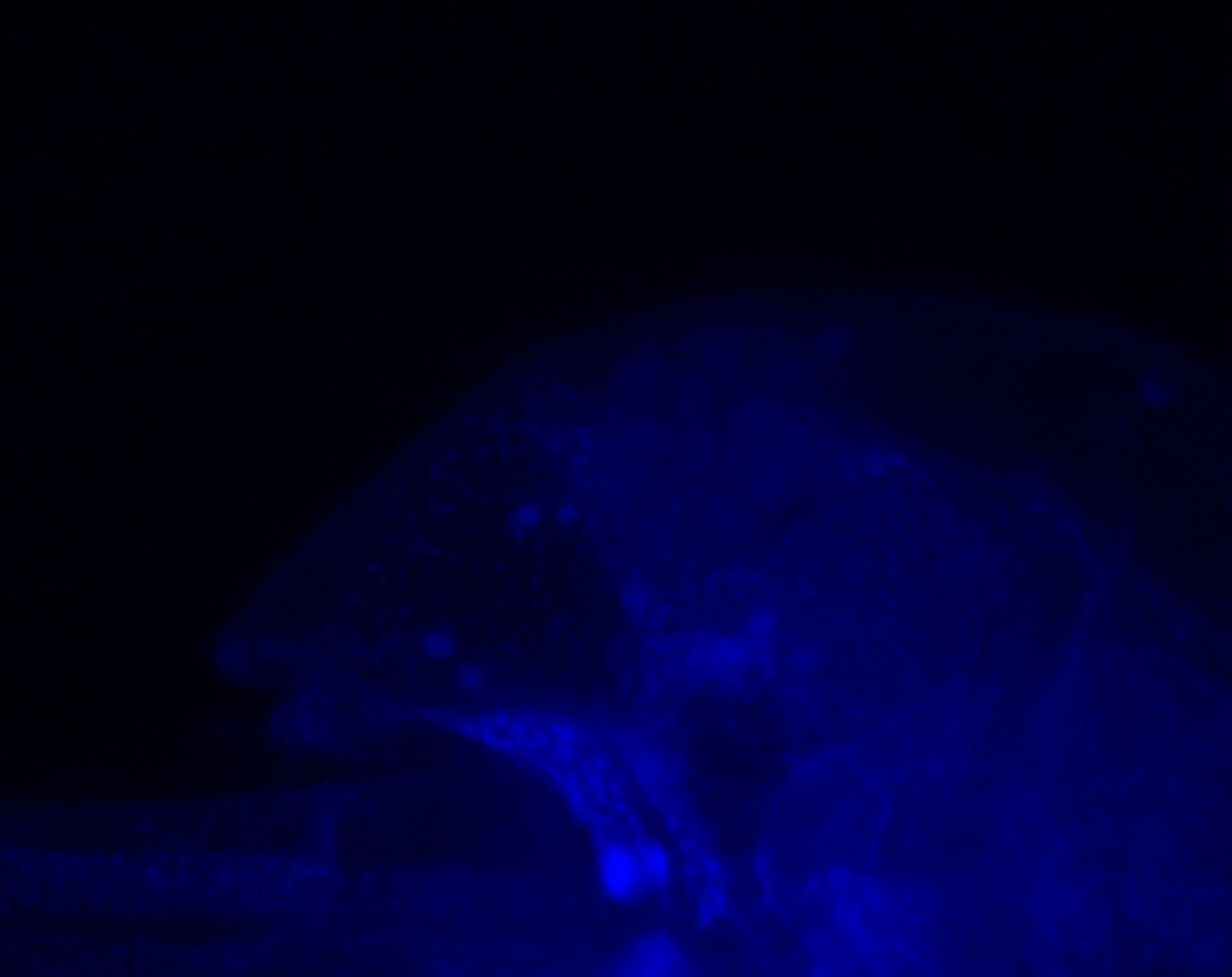
For red: *[black first]- enter 6 zeros, then ok; and on the same screen:
[red second] enter f81106, then ok for second screen and another ok for first screen
*if this doesn't work, do red first

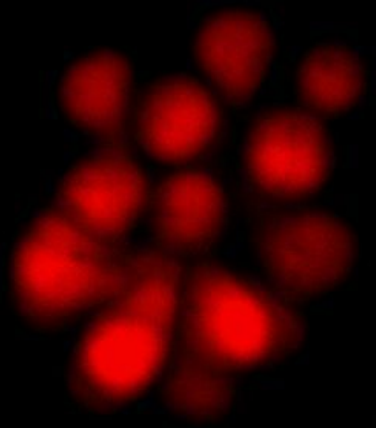
To merge photos:
New file
Preset- US paper (or letter)
Image resolution- make 300 (<300- writing not clear; 600 is good too)
Select, copy and paste both images to new file
View bigger to see shape better

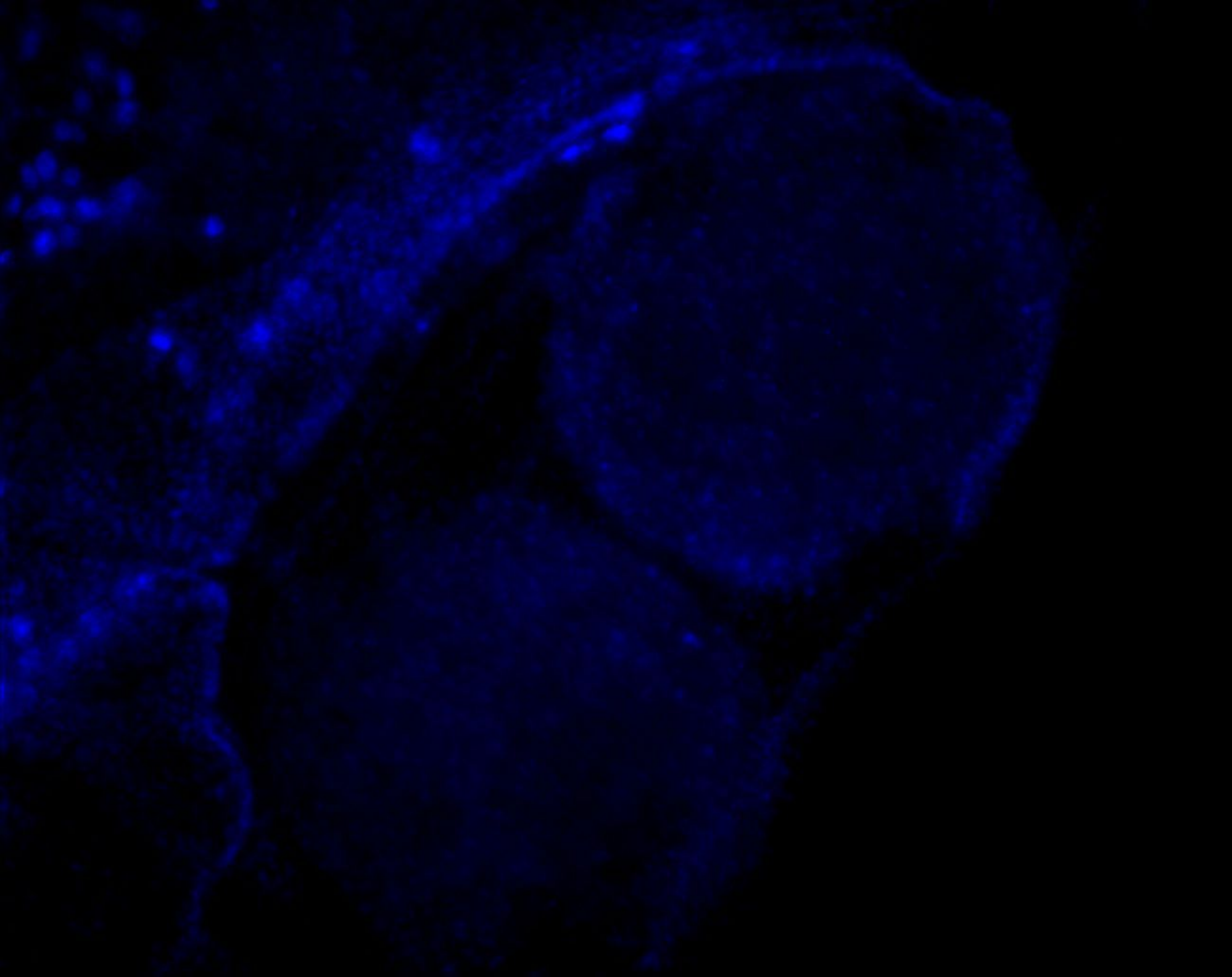
Put horizontal and vertical rulers to make sure images are aligned
Put rulers for part of image you like to present, e.g.:
Image length: 2
Image width: 3.5
Make sure you highlight that layer in the toolbox on the right side
Select the 2 x 3.5 area
Layer mask (to crop photo) –reveal selection

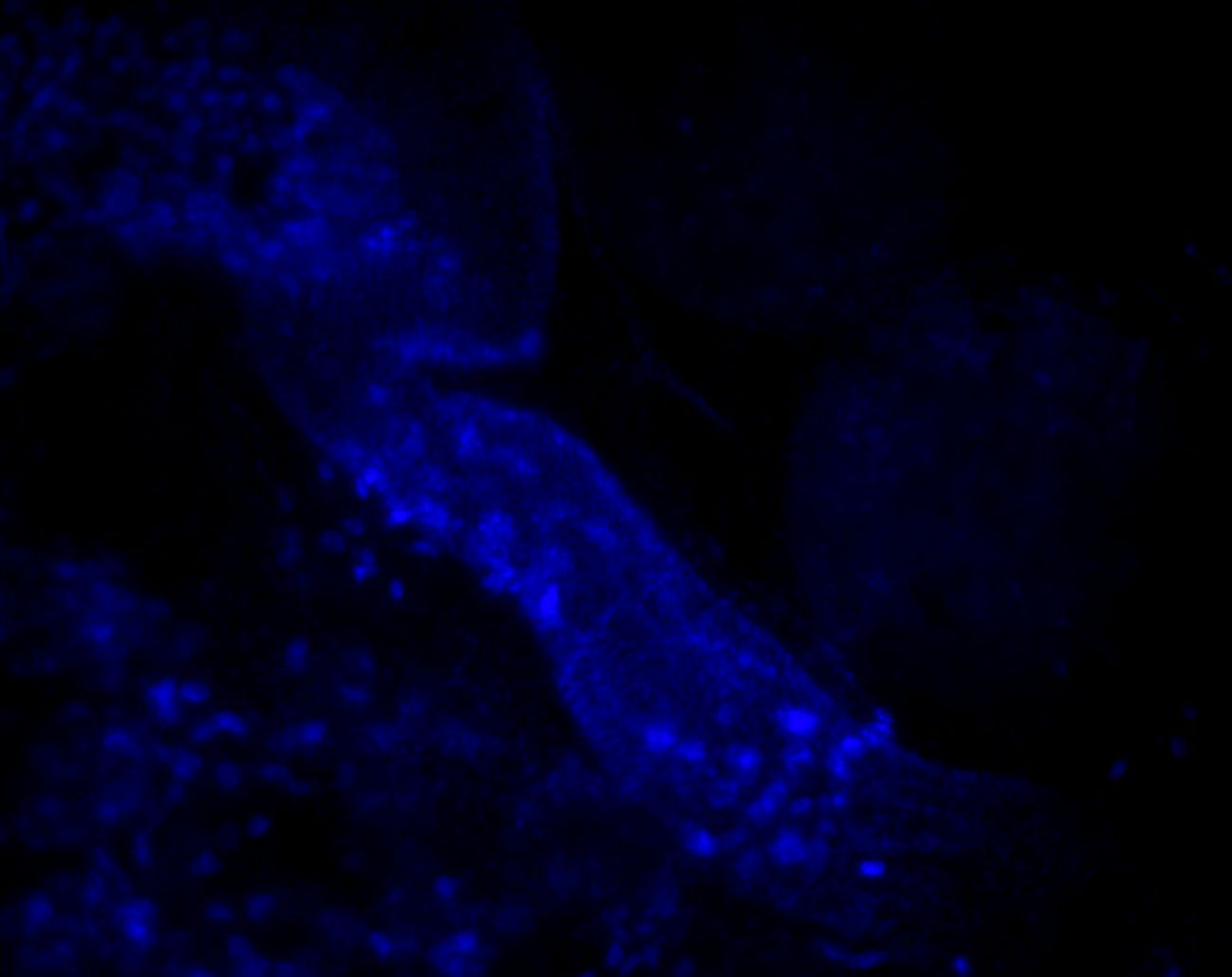
To merge:
*Duplicate images to be used to create a merged image (to have red/blue, and merged)
*it duplicates over the layer so just move the duplicate to the side
Check toolbox on right for layer type for the merged image:
*Normal for one image/layer – the less dominant color
Screen for other image/layer – the more dominant color
*one has to be screen to see overlay

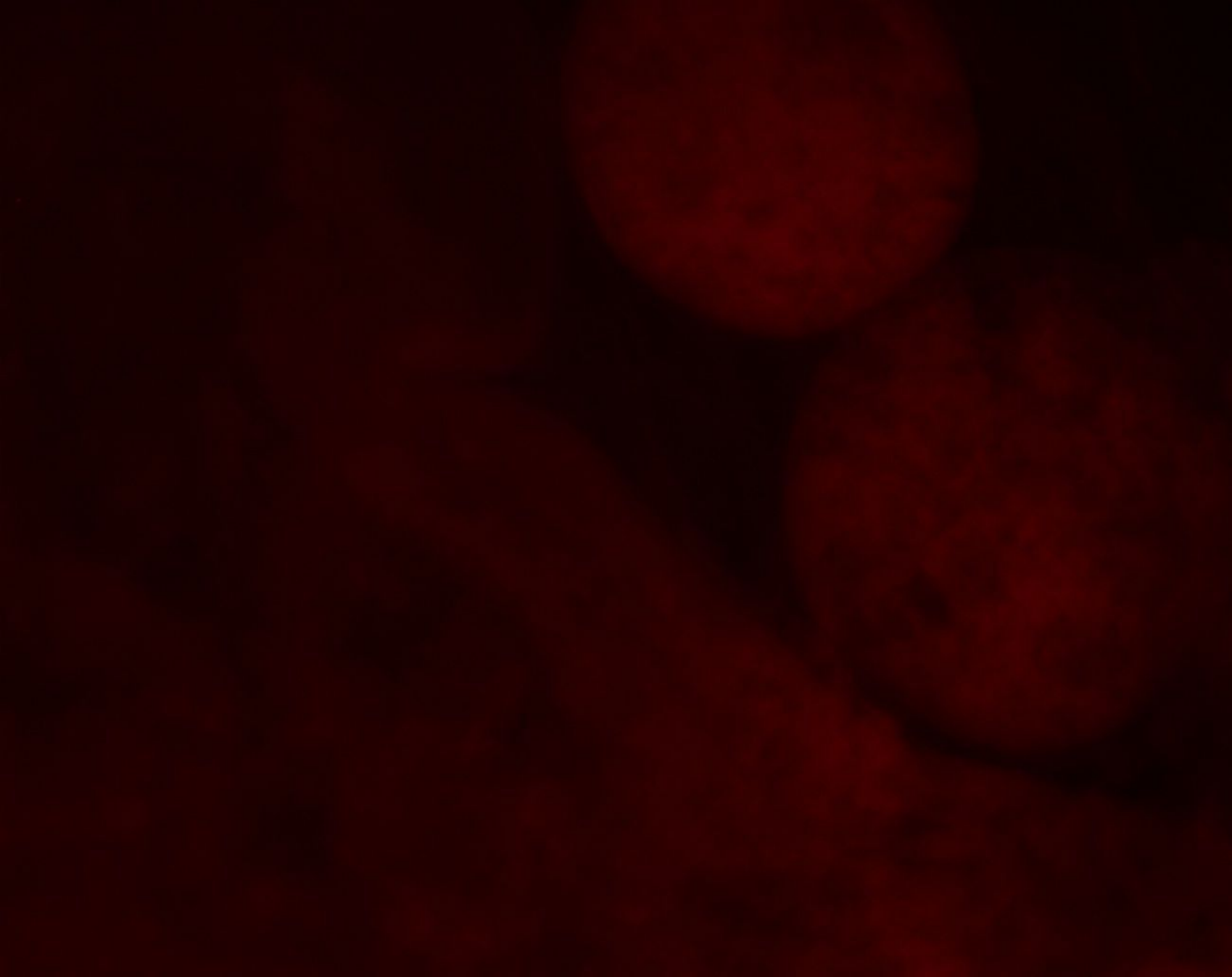


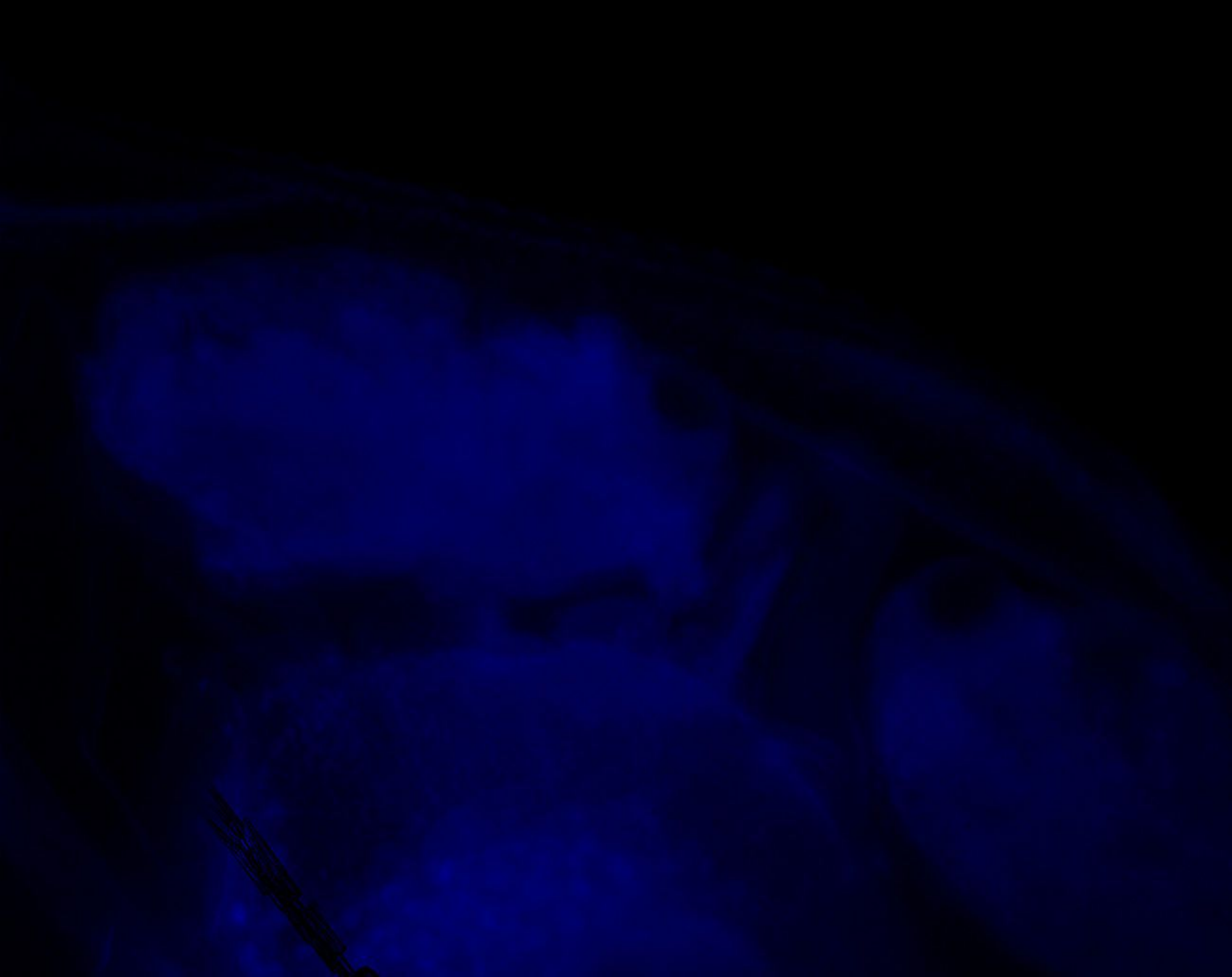


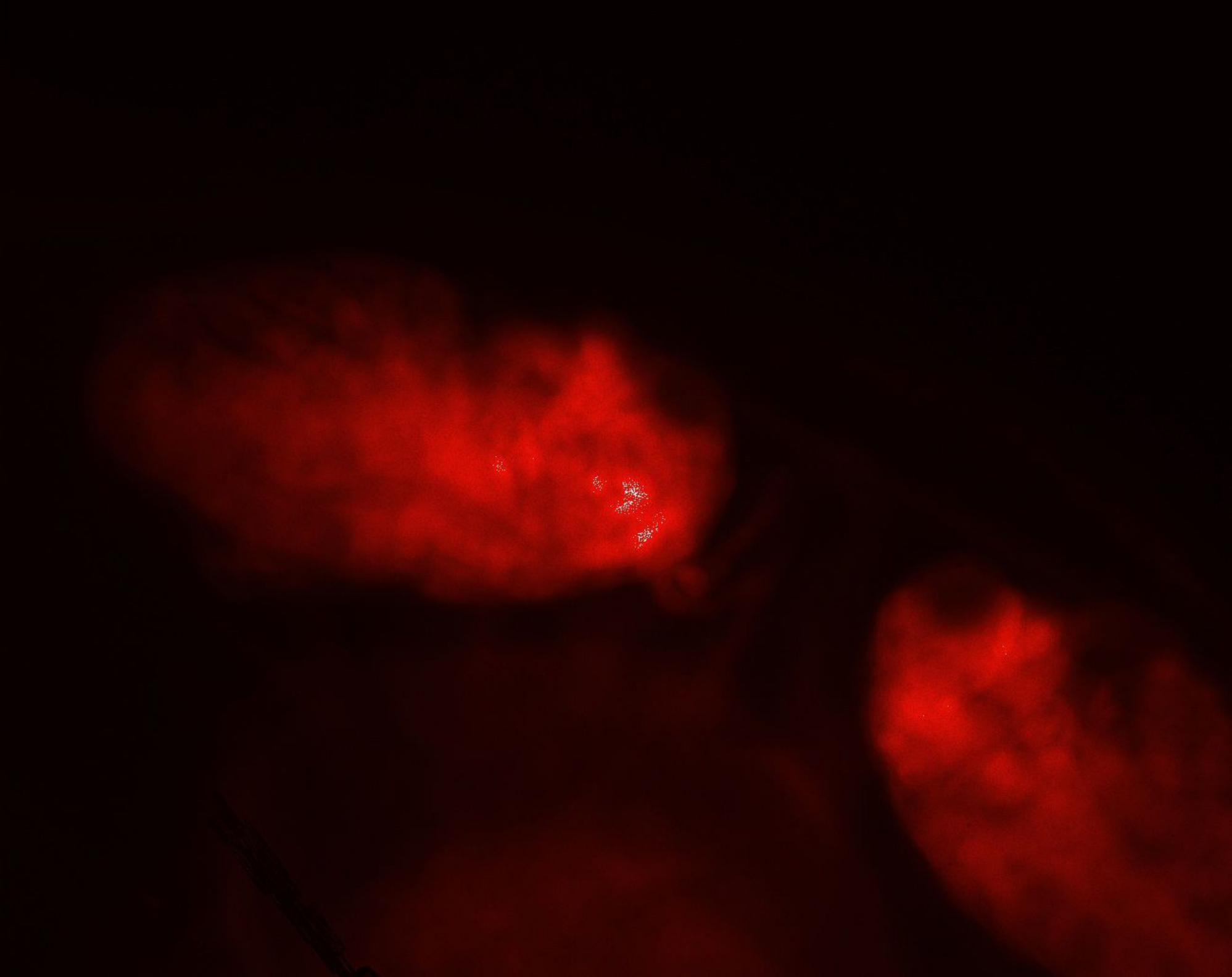


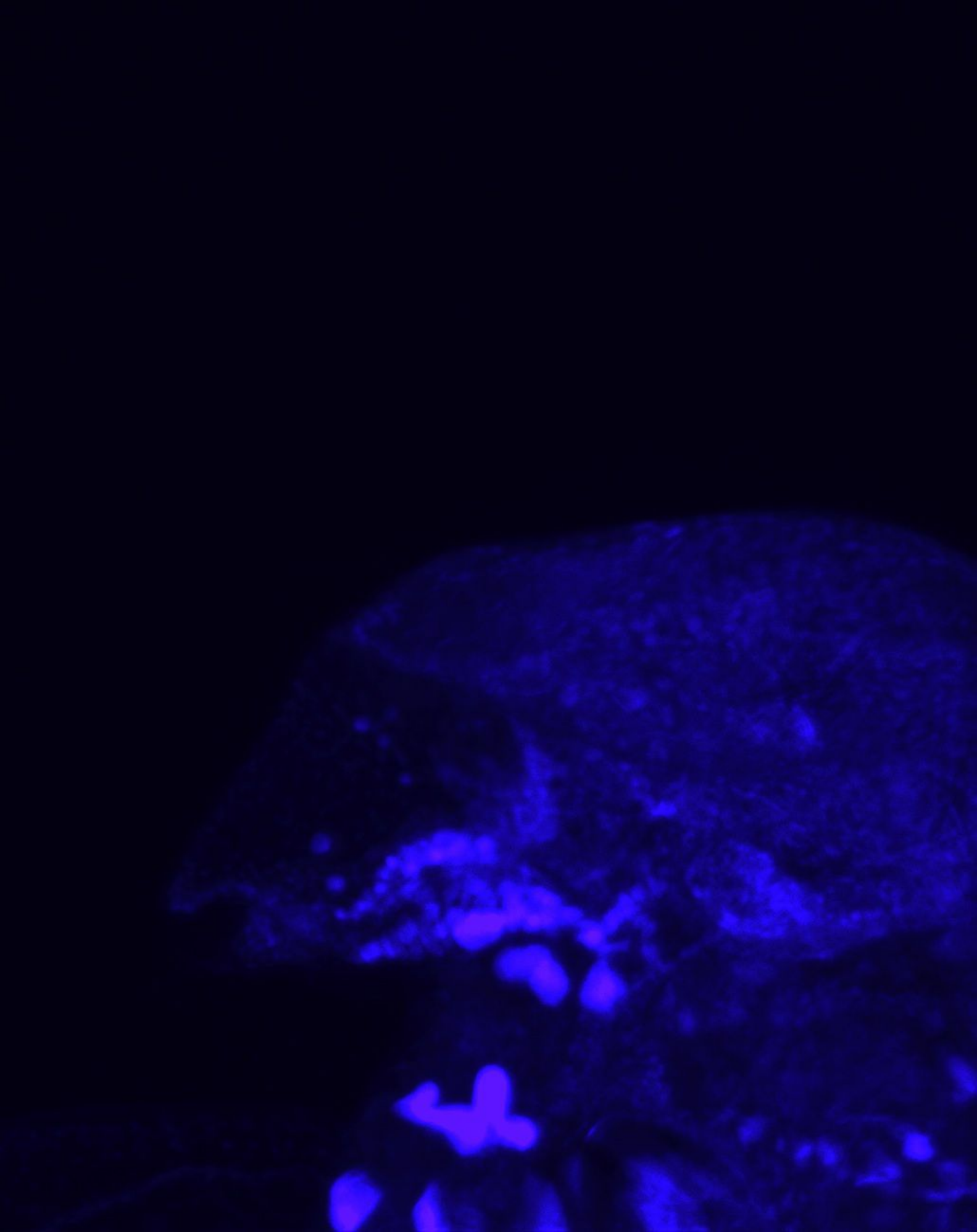


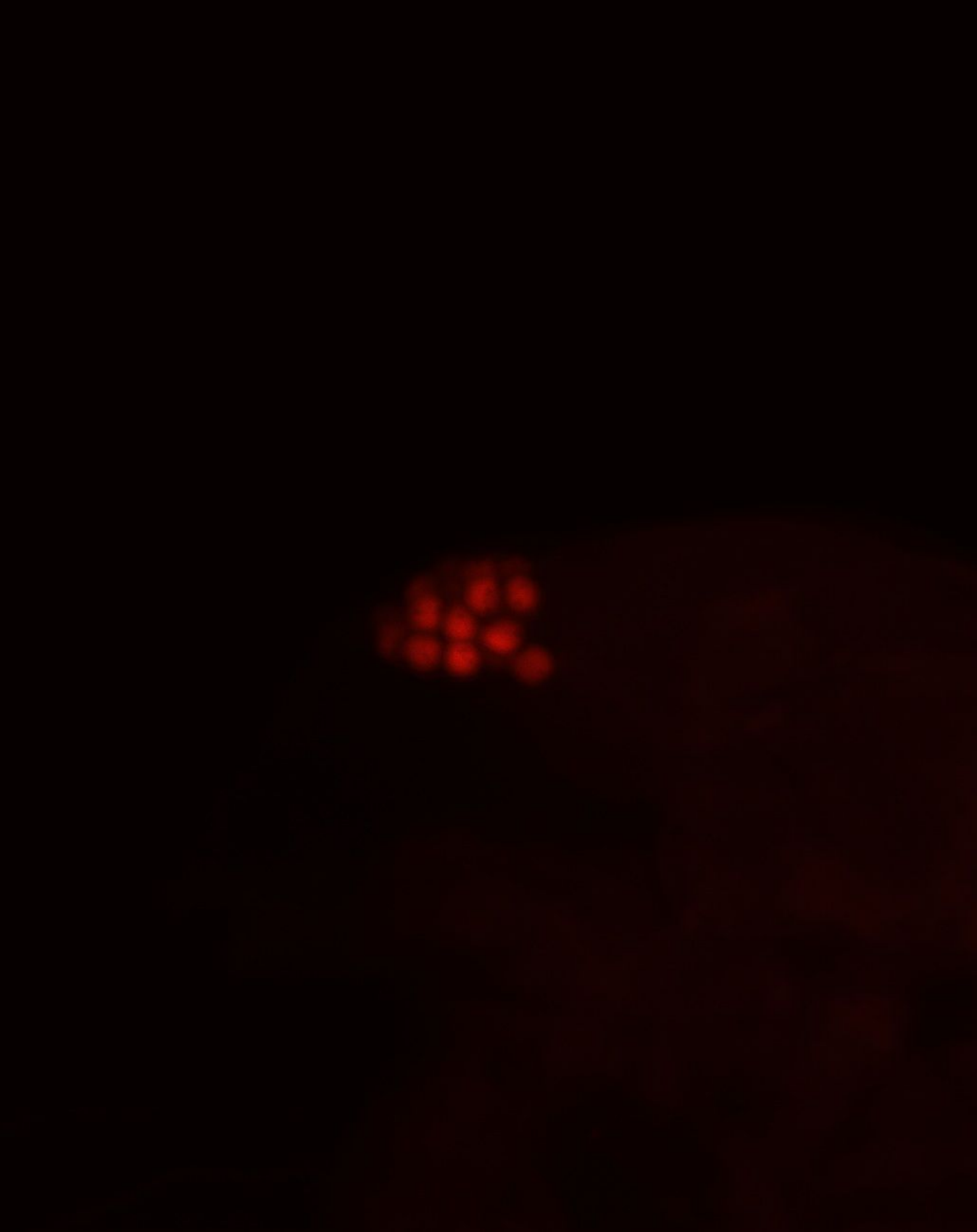


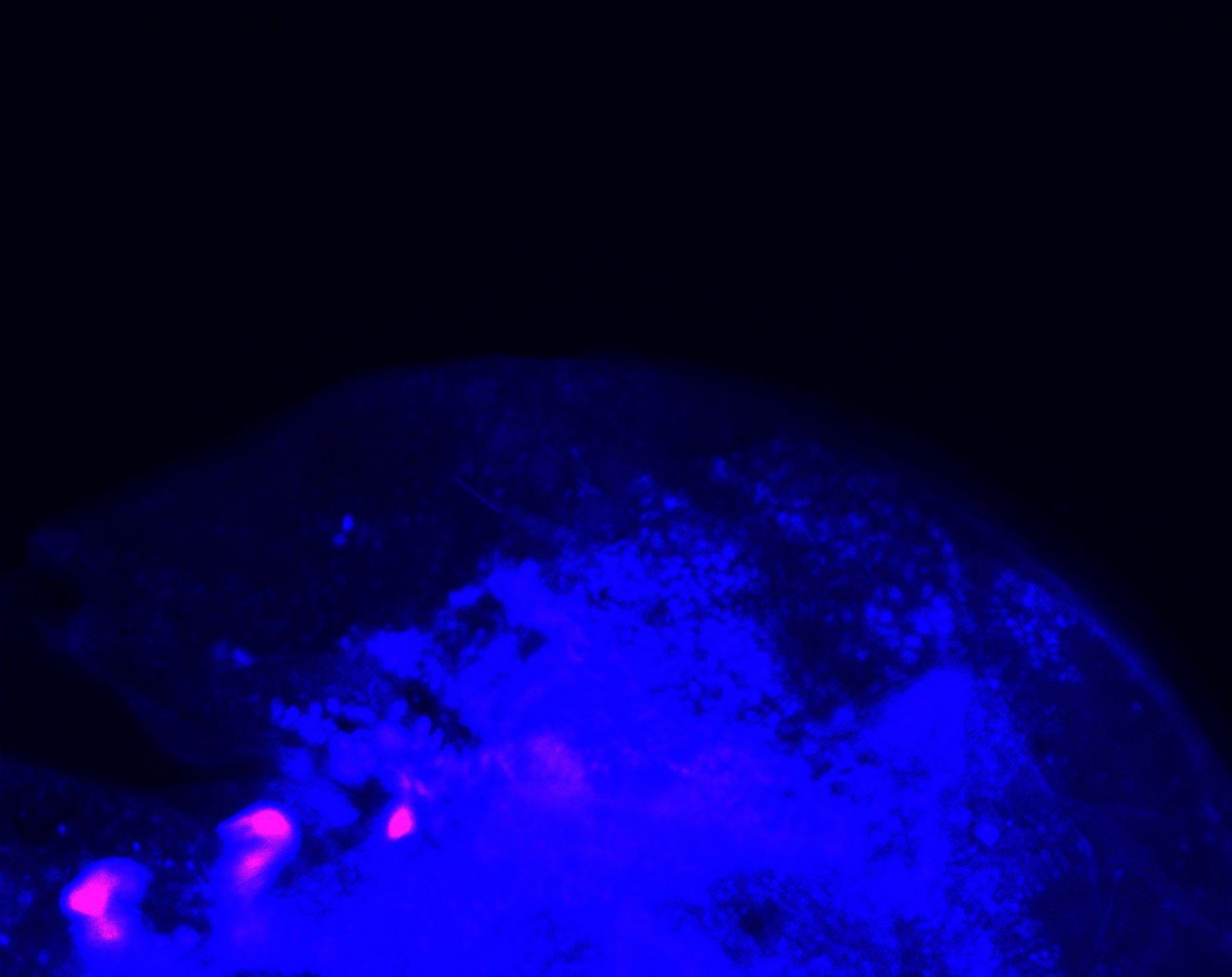


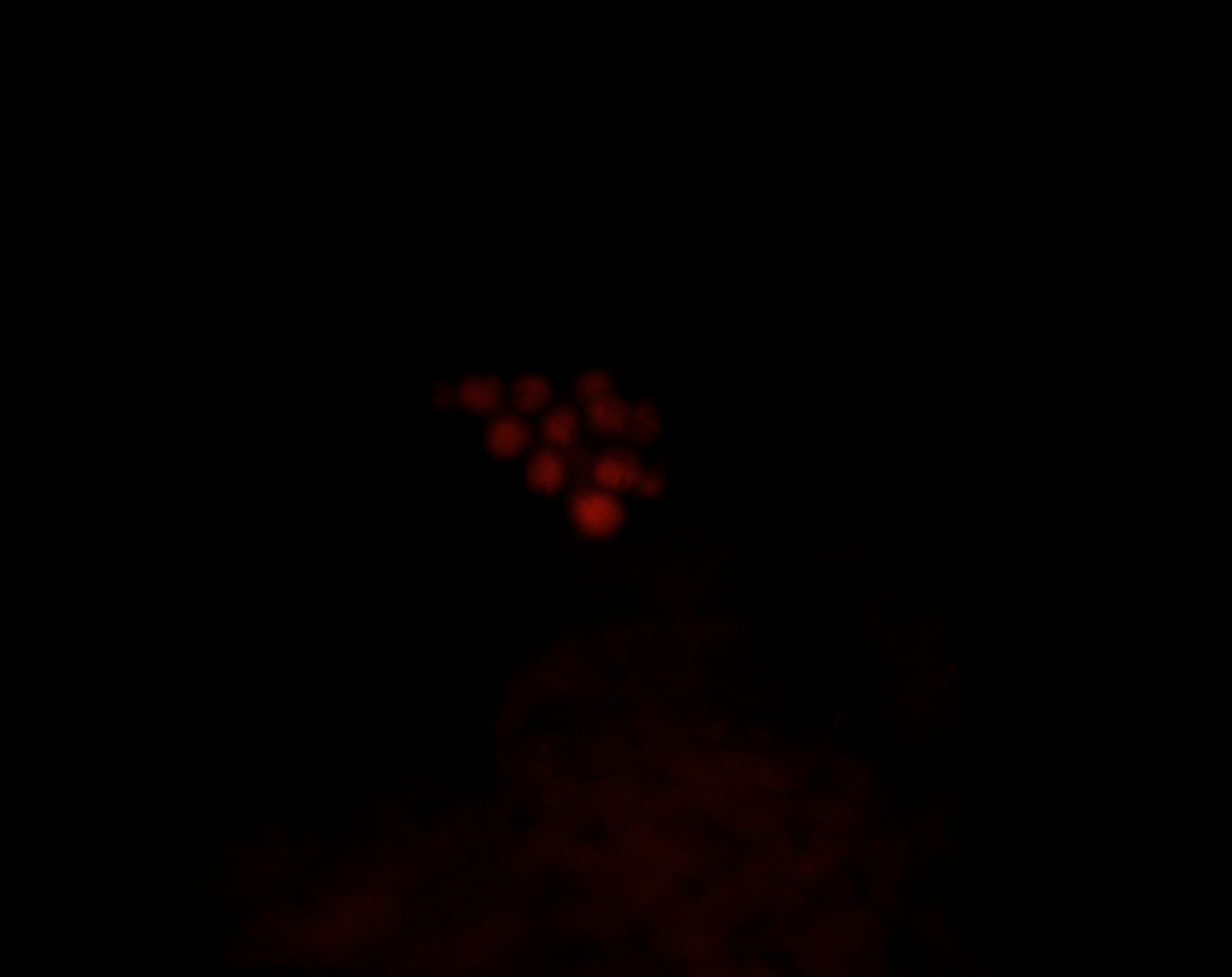


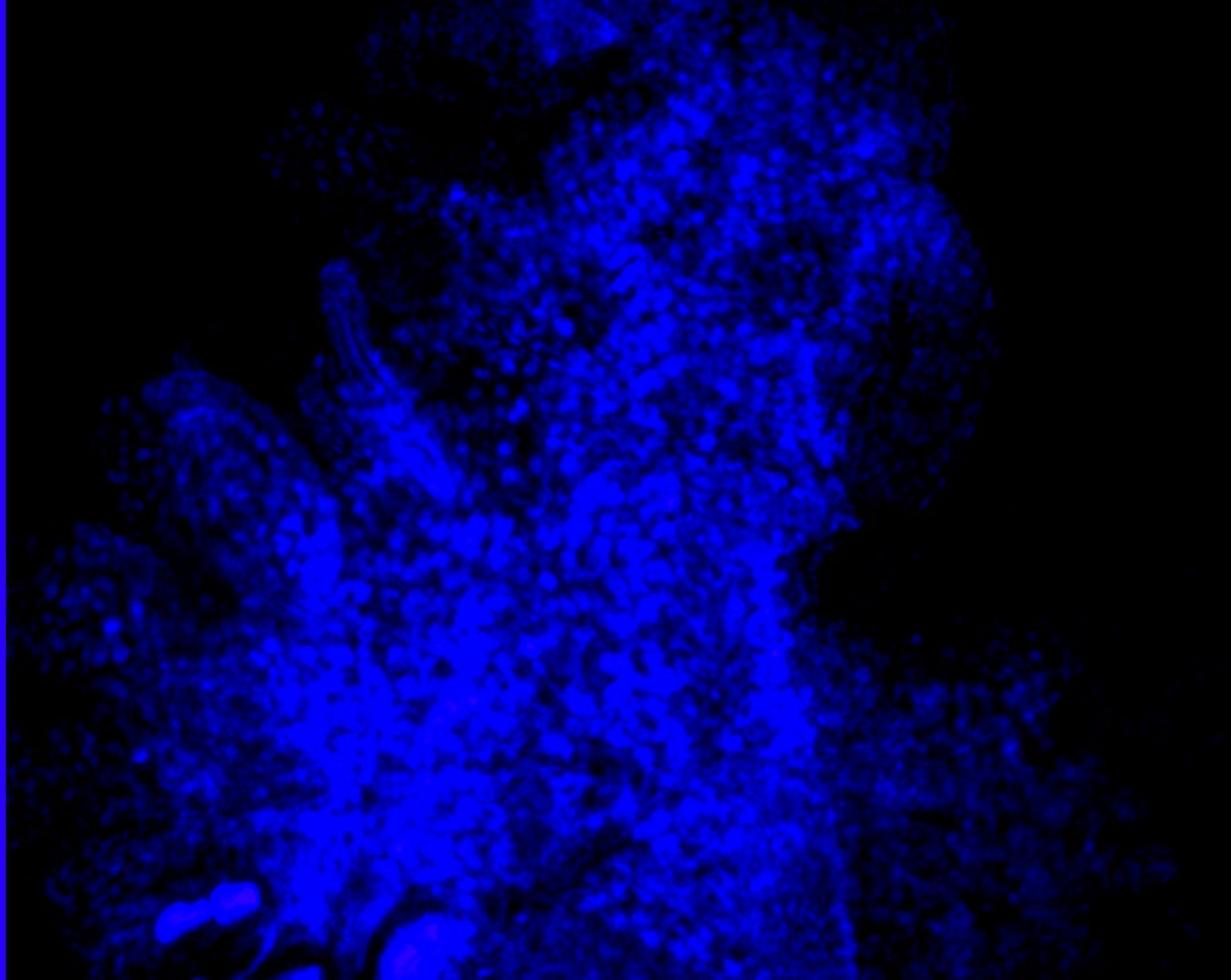


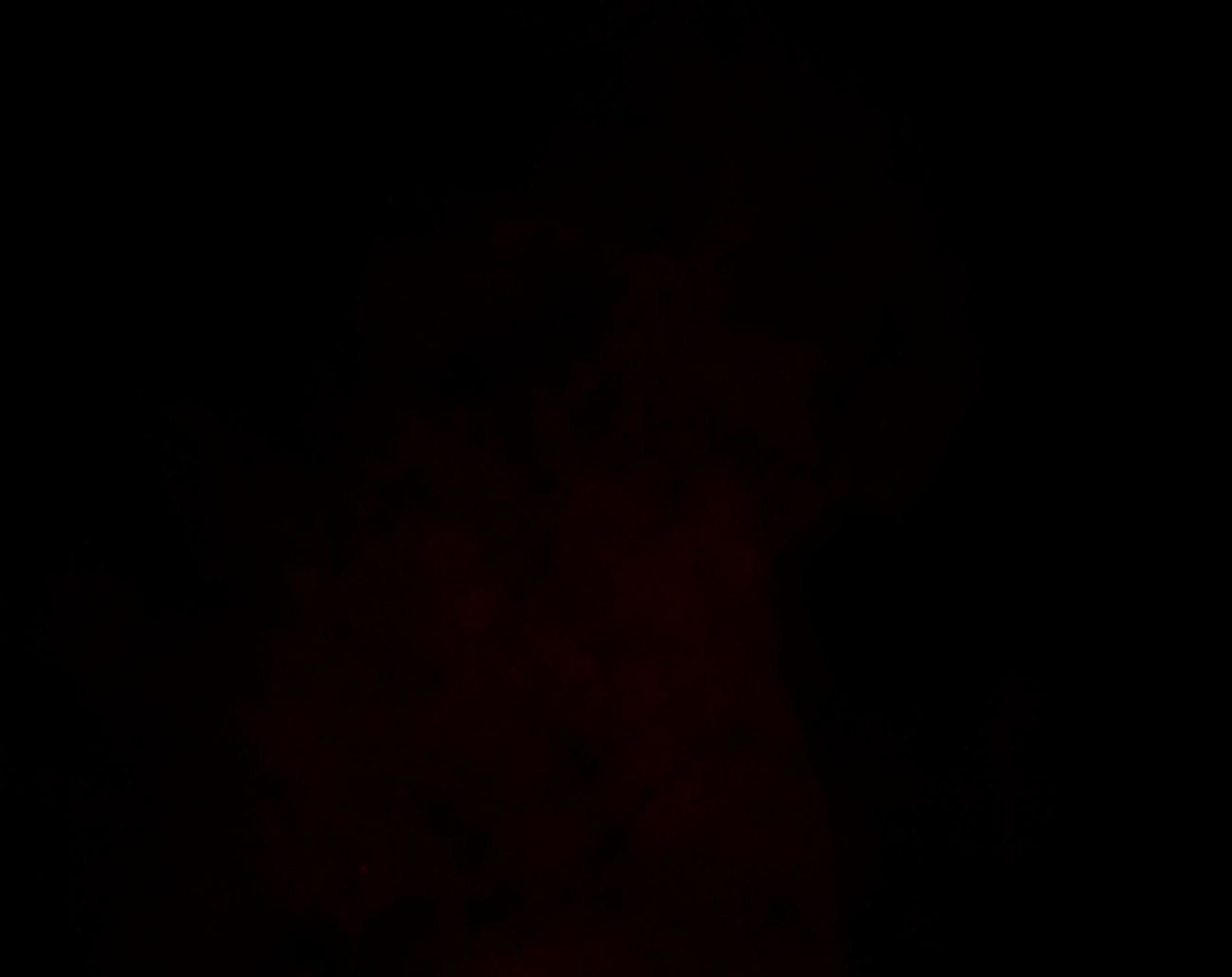


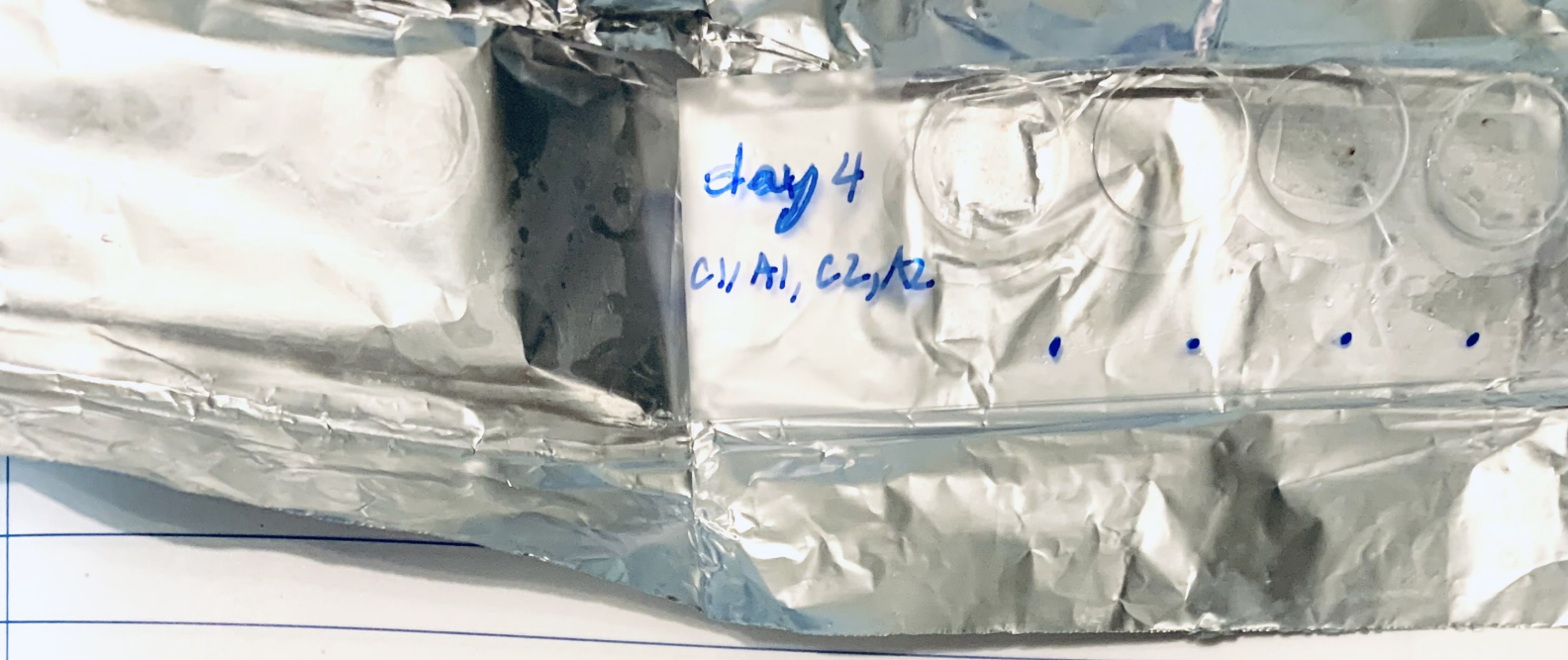


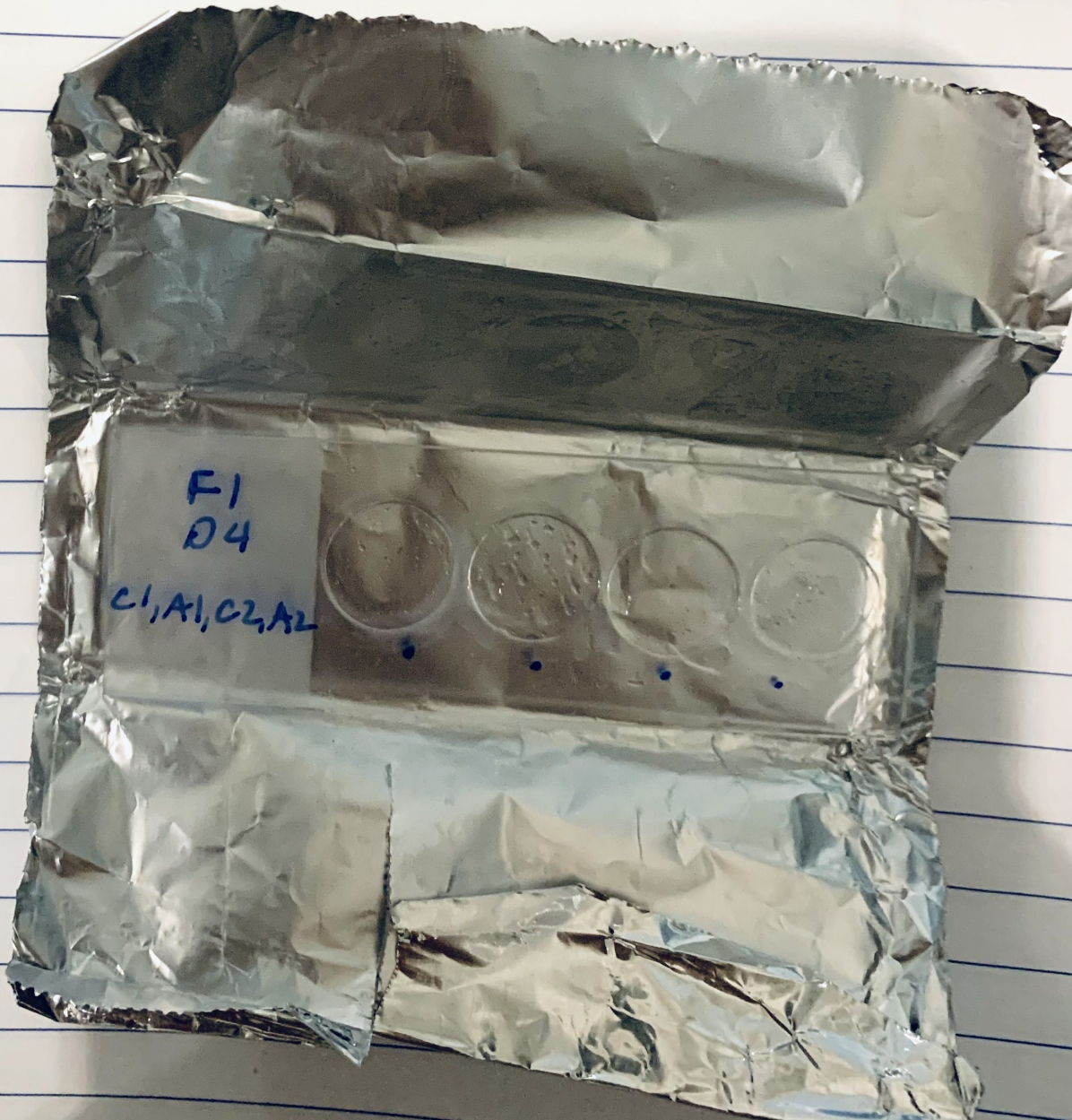









day 4
C1, A1, C2, A2


F1
D4
C1, A1, C2, A2

day 1

C1, A1, C2, A2

day 2

C1, A1, C2, A2

day 7

C1, A1, C2, A2