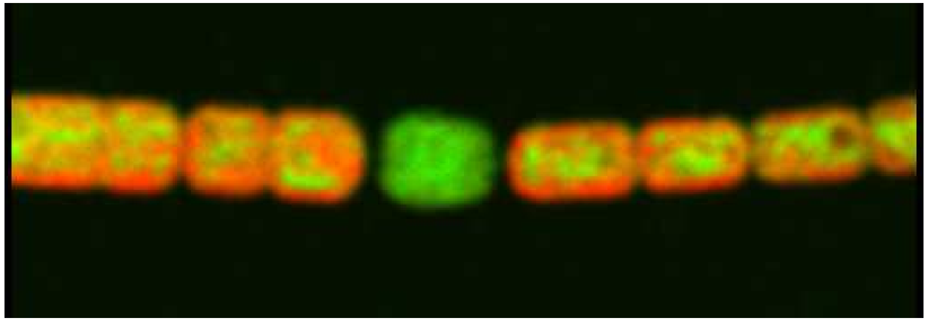
Intercellular molecular exchange in filamentous cyanobacteria

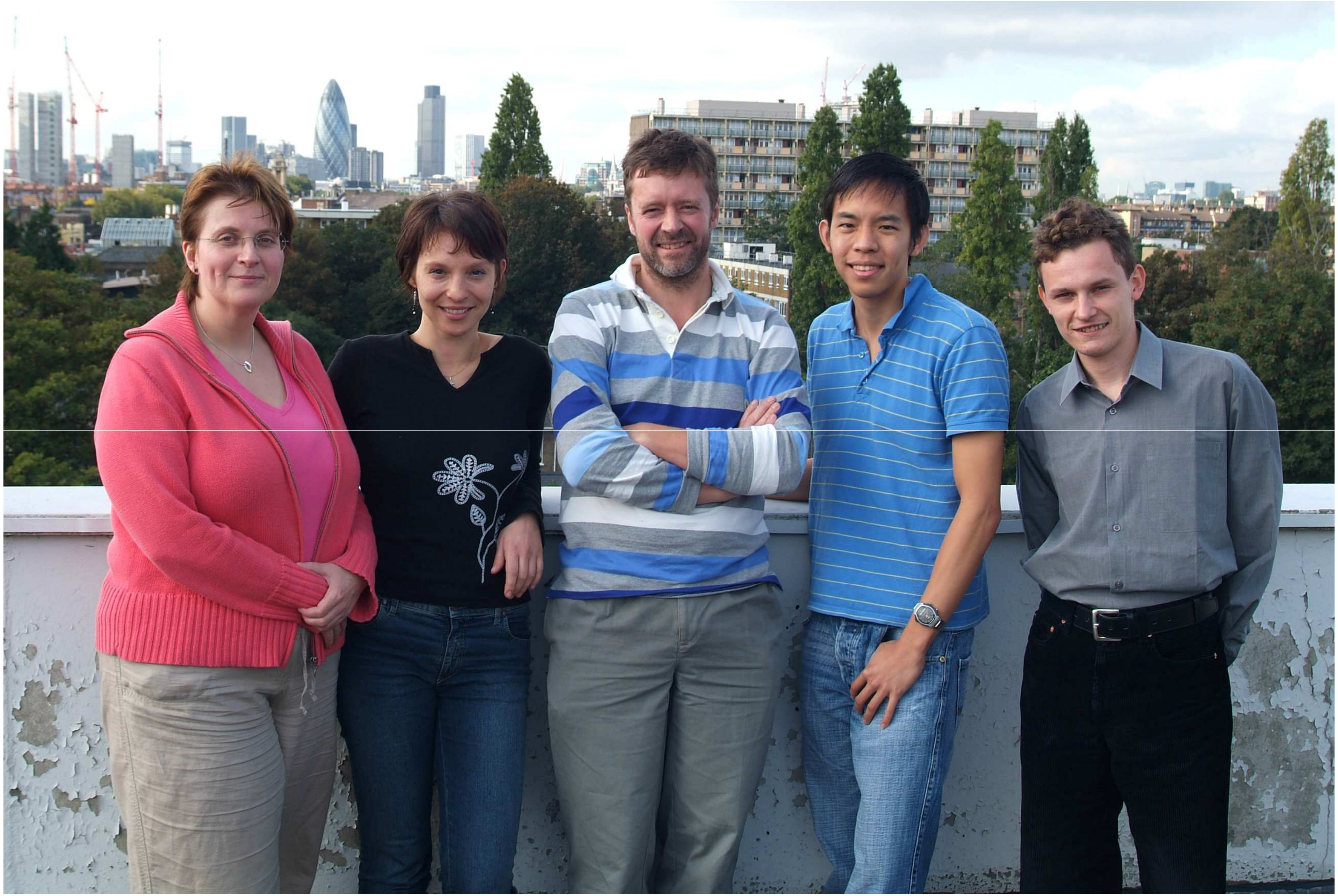


Conrad Mullineaux, Anja Nenninger, Hajara Khanum
Queen Mary, University of London

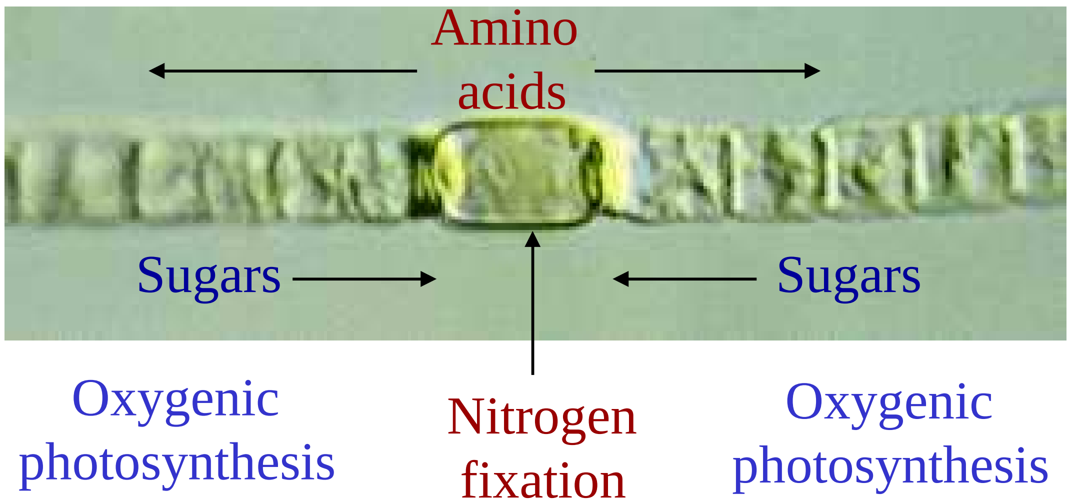
Vicente Mariscal, Antonia Herrero, Enrique Flores
IBVF, Sevilla

David G. Adams
University of Leeds

EMBO J *in press* (2008)



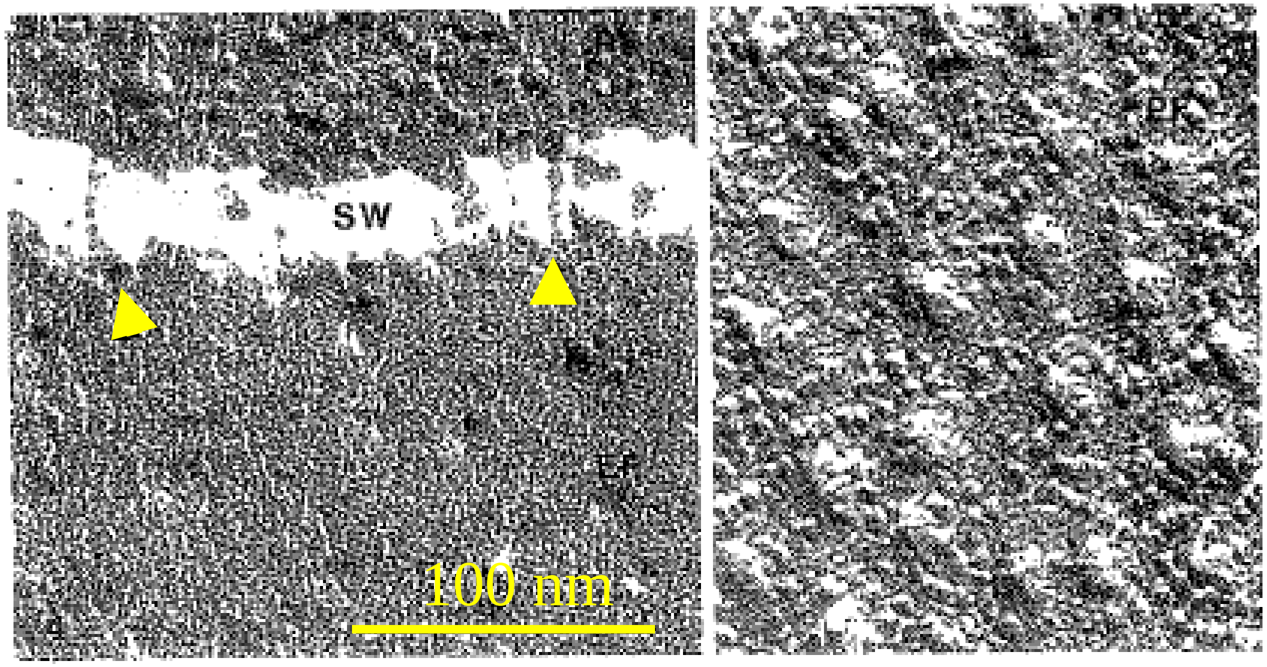

SANTA CRUZ


Amino
acids
Sugars
Sugars
Oxygenic
photosynthesis
Nitrogen
fixation
Oxygenic
photosynthesis

How do metabolites exchange between cells?

1. “Microplasmodesmata”?

Giddings and Staehelin (1978) Cytobiologie 16, 235-249



Anabaena cylindrica

external diameter about 20 nm

200-300 at Veg-Veg junctions, about 50 at Het-Veg junctions

How do metabolites exchange between cells?

1. "Microplasmodesmata"?

Anabaena 7120 (Iris Maldener/Enrique Flores)

How do metabolites exchange between cells?

2. via the periplasm?

Opinion TRENDS in Microbiology Vol.14 No.10

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Is the periplasm continuous in filamentous multicellular cyanobacteria?

Enrique Flores[1], Antonia Herrero[1], C. Peter Wolk[2] and Iris Maldener[3]

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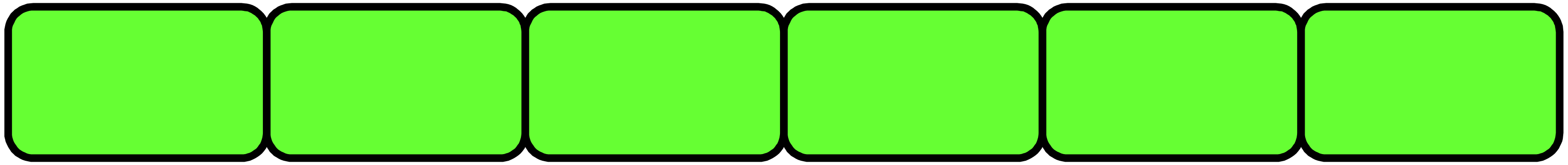
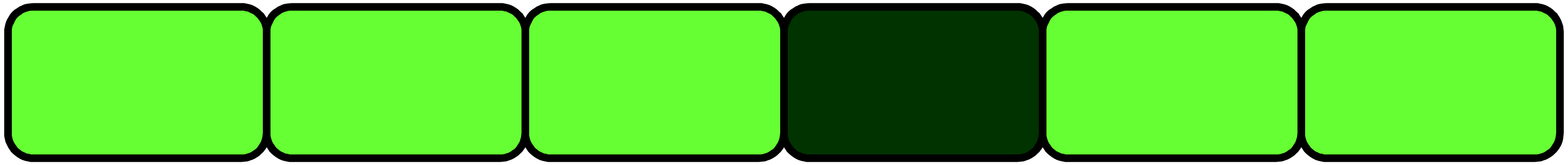
Trends in Microbiology 14 (2006) 439-442

Probing molecular exchange between cells by Fluorescence Recovery after Photobleaching (FRAP)

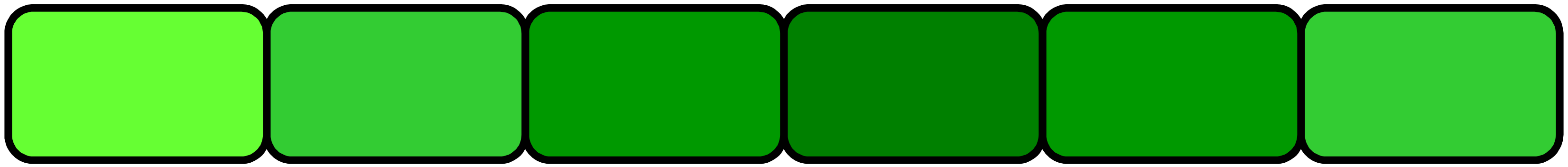
1. Load the cytoplasm with a fluorescent molecule, observe in laser-scanning confocal

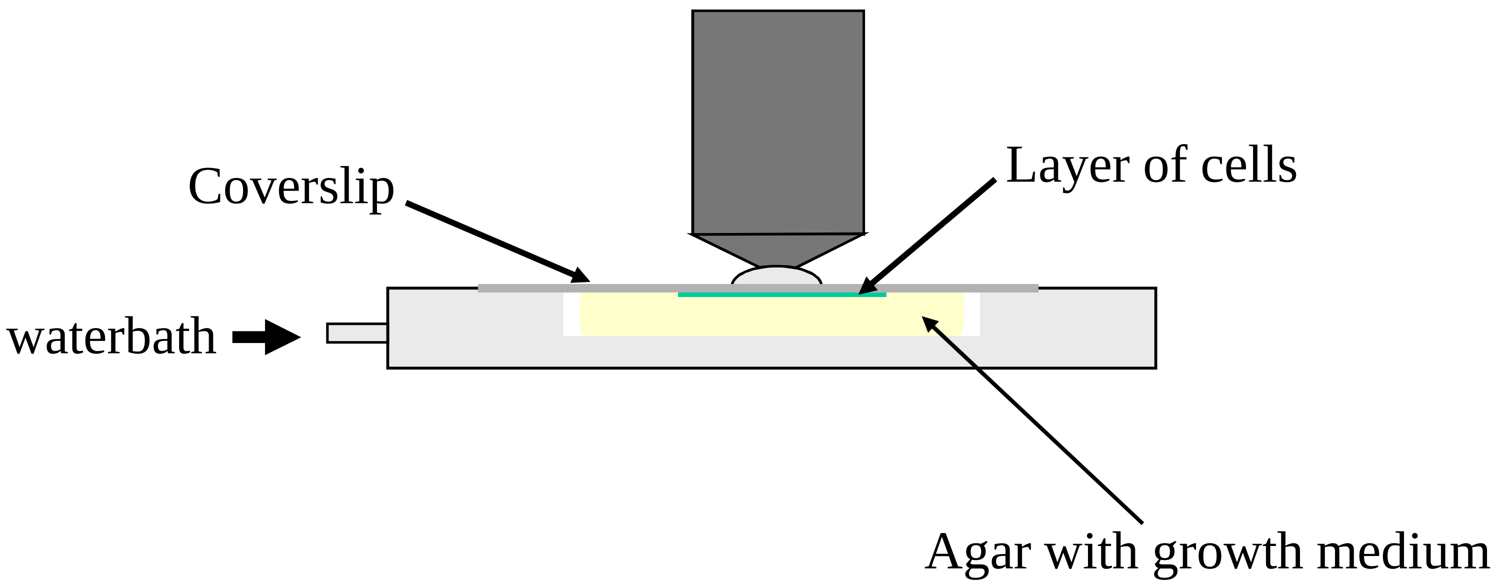


2. Bleach the fluorescence in one cell

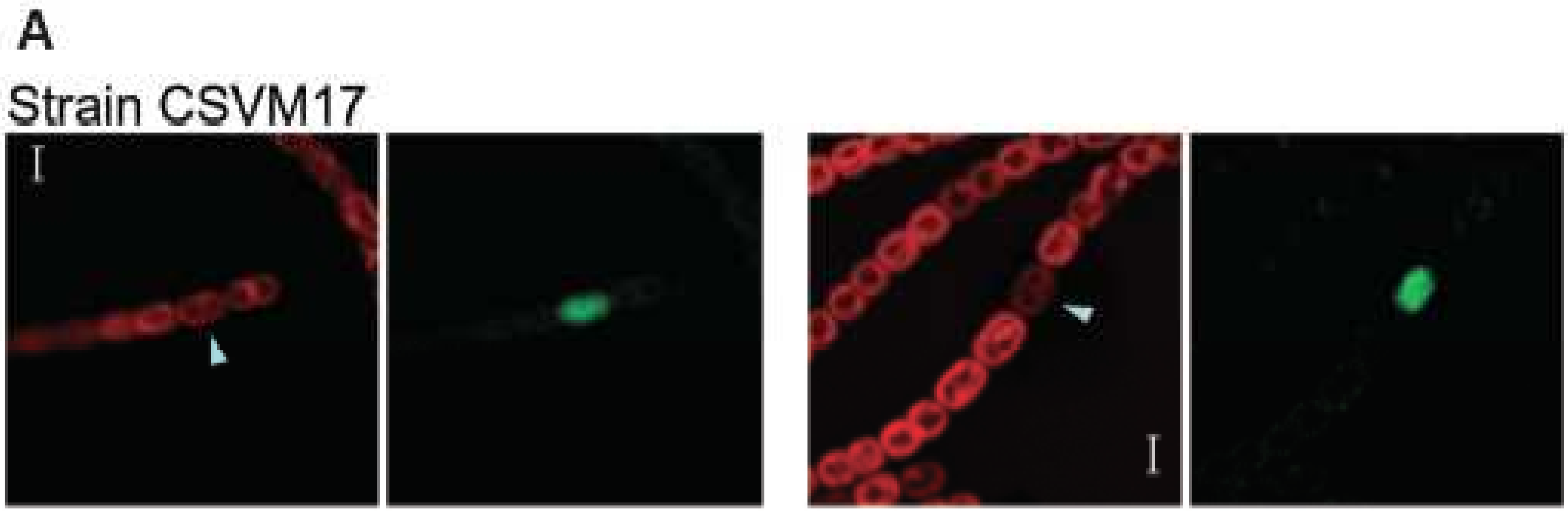


3. What happens next?




Coverslip
Layer of cells
waterbath
Agar with growth medium

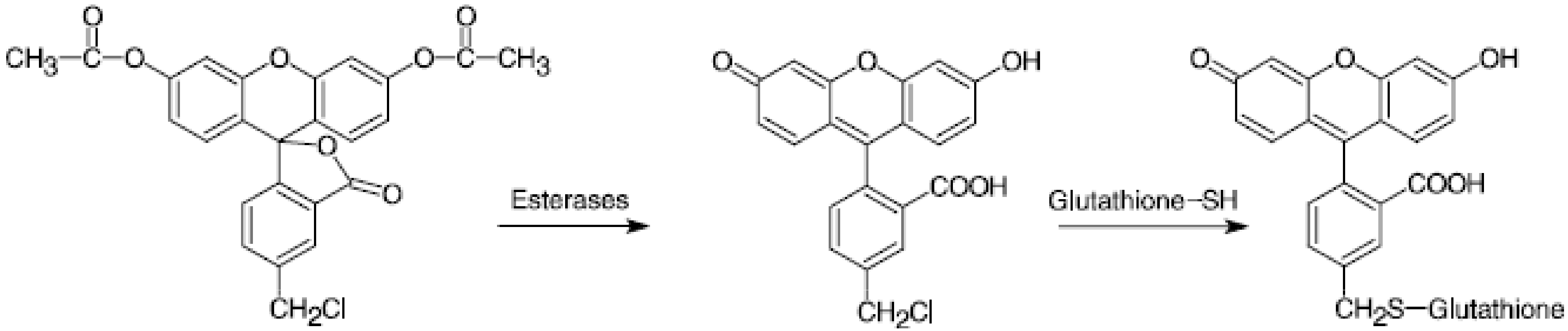
GFP cannot move from cytoplasm to cytoplasm



GFP expressed in a pro-heterocyst of *Anabaena* 7120

Mariscal et al. (2007) Mol Microbiol 65, 1139-1145 but what about smaller molecules?

1. CellTracker Green CMDFA (Invitrogen)

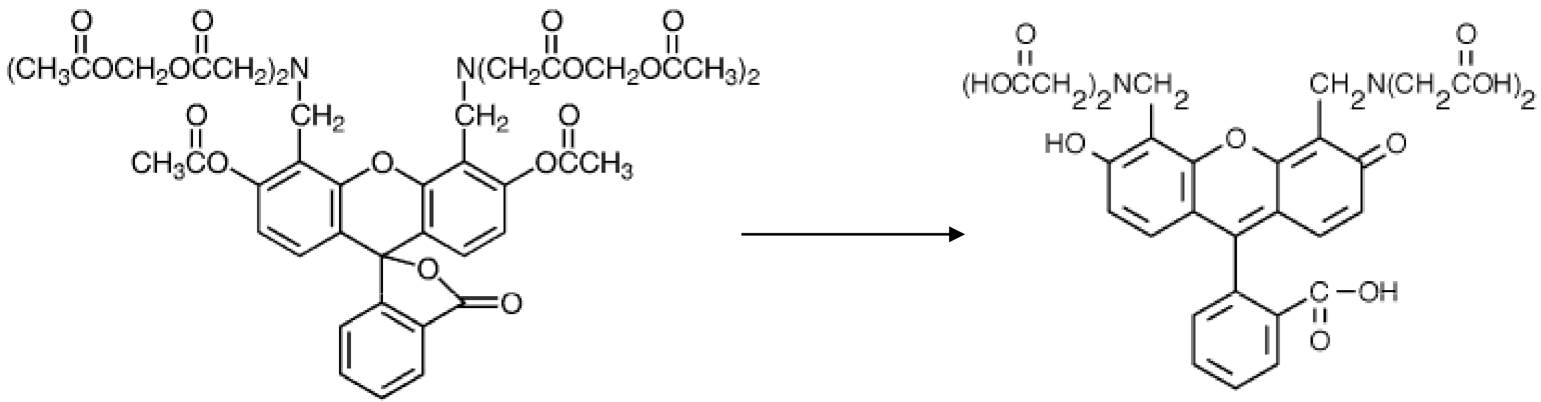


non-fluorescent
membrane-permeable

green fluorescence
hydrophilic

but what about smaller molecules?

2. Calcein-AM (Invitrogen)



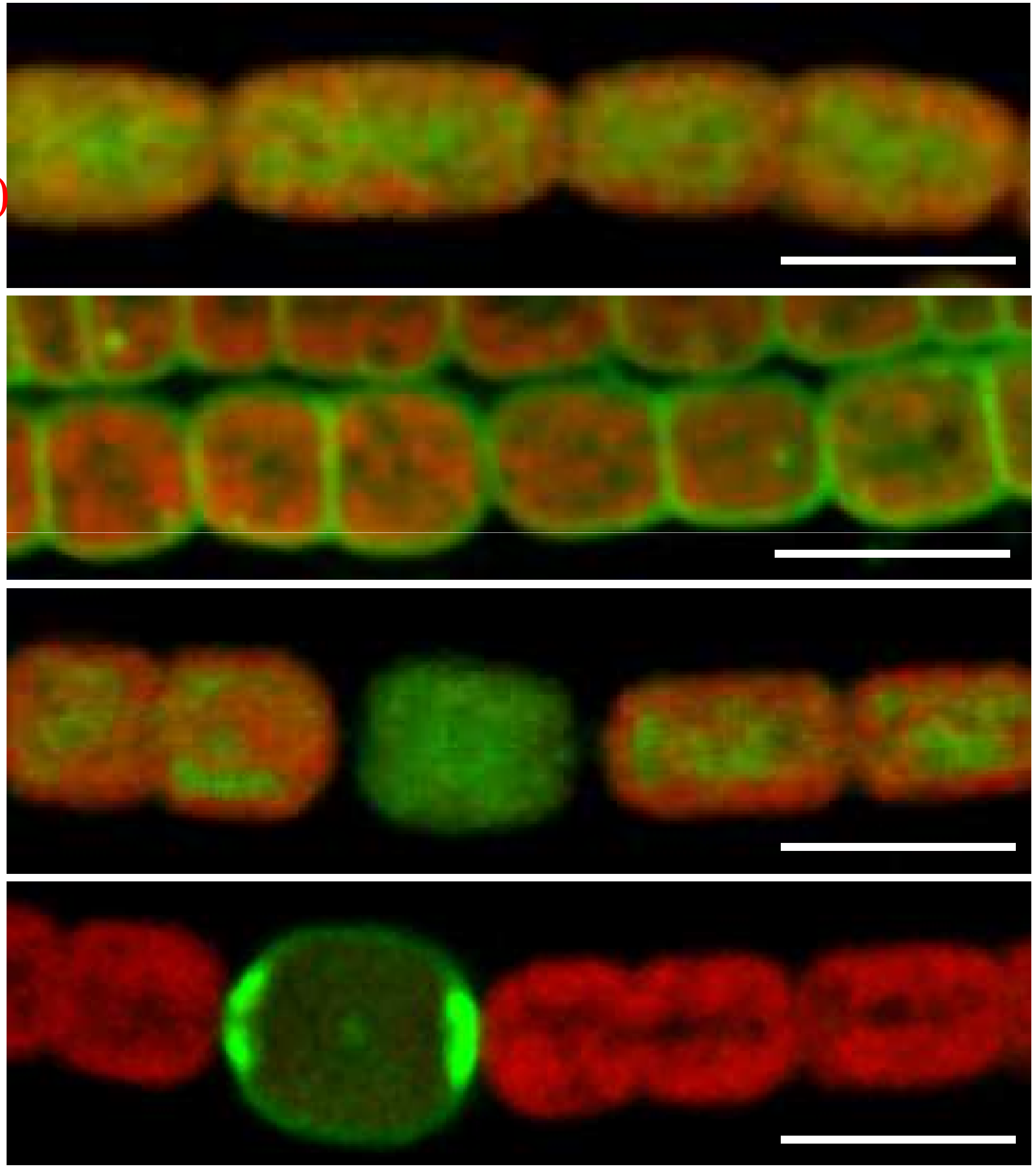
non-fluorescent
membrane-permeable

green fluorescence
hydrophilic
623 Da

Calcein in *Anabaena cylindrica* (PCC 7122)

= thylakoids (Chlorophyll fluorescence)

= dye (Calcein or BODIPY)



Nitrate
Calcein

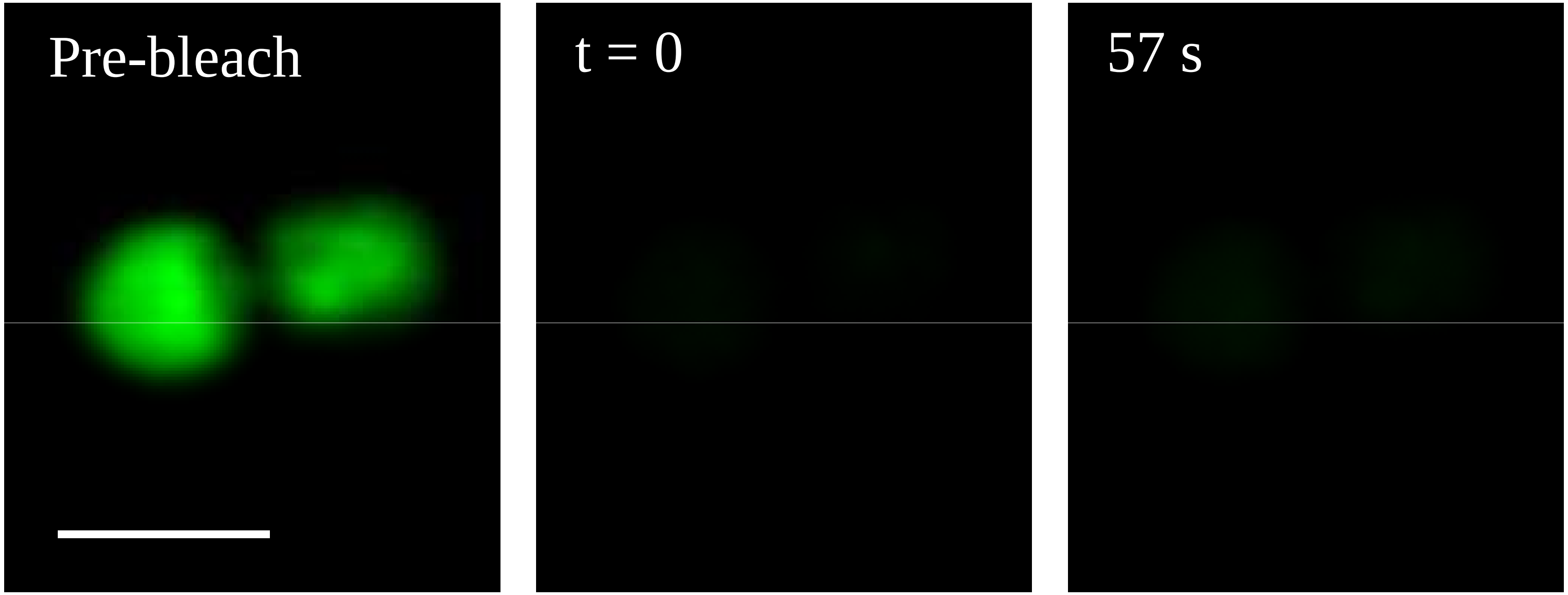
Nitrate
BODIPY FL C_{12}

N_2
Calcein

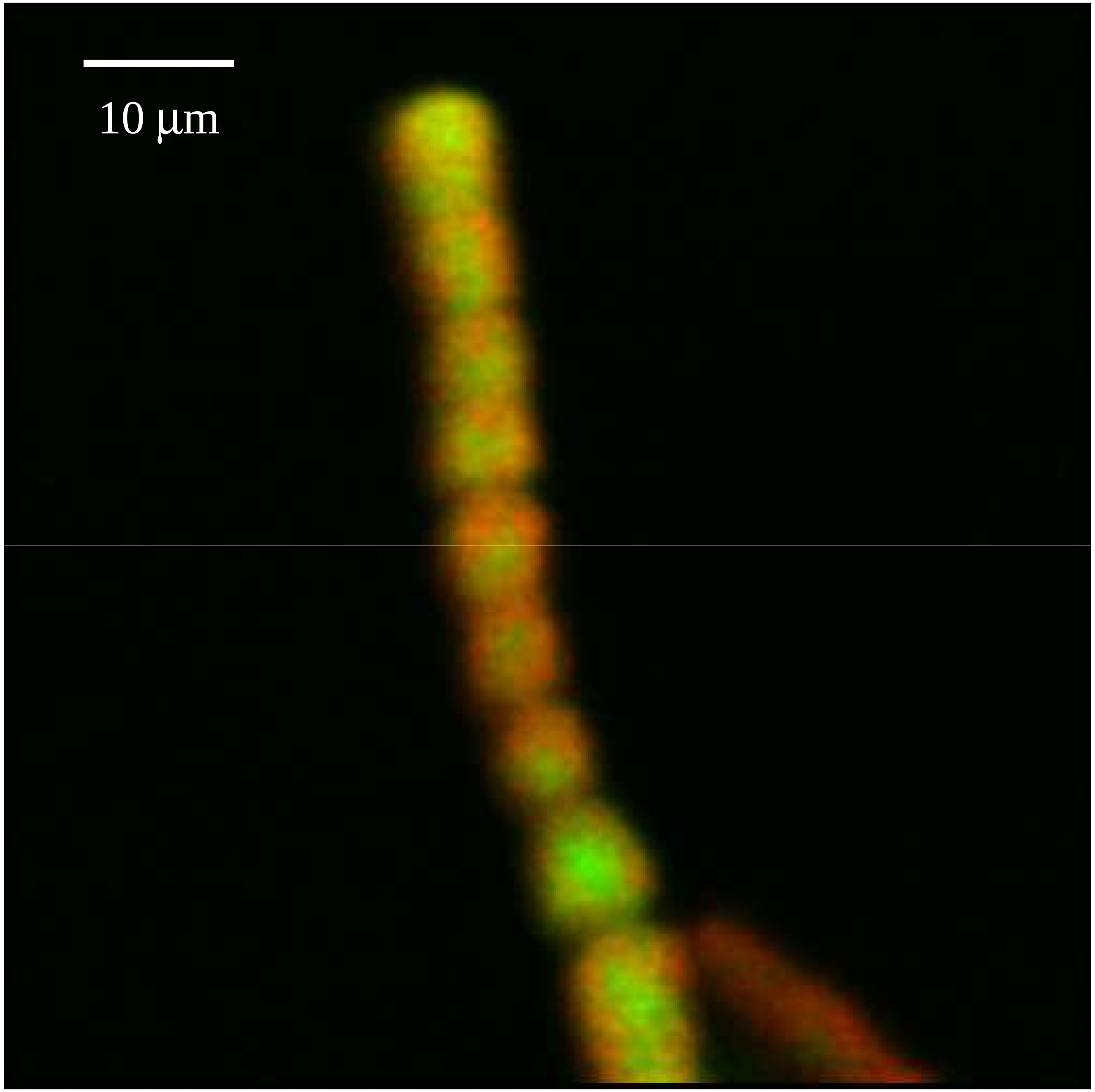
N_2
BODIPY FL C_{12}

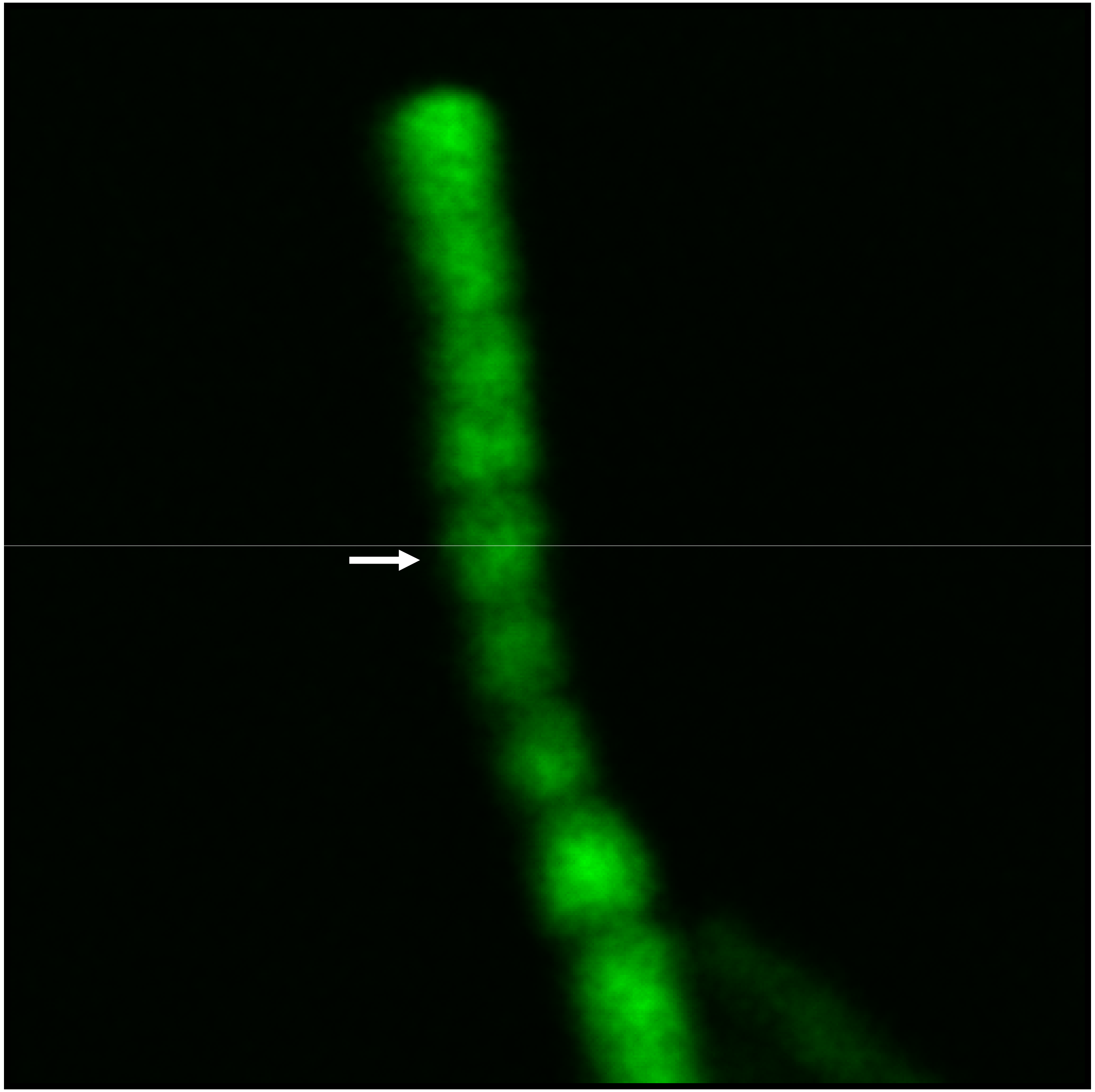
Scalebars
10 µm

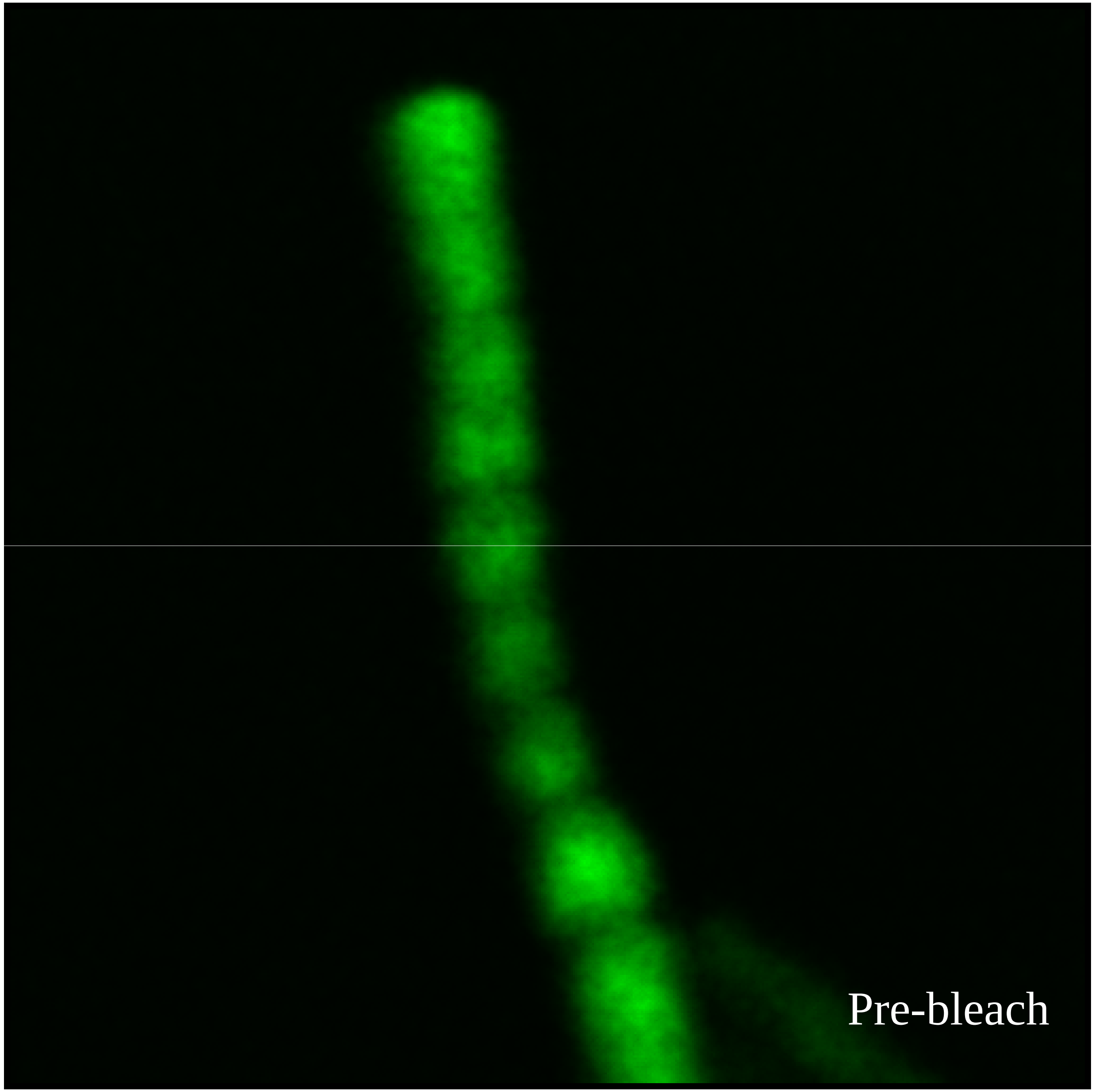
Photochemical bleaching of calcein is irreversible



Calcein FRAP in *Anabaena cylindrica* (nitrate-grown)



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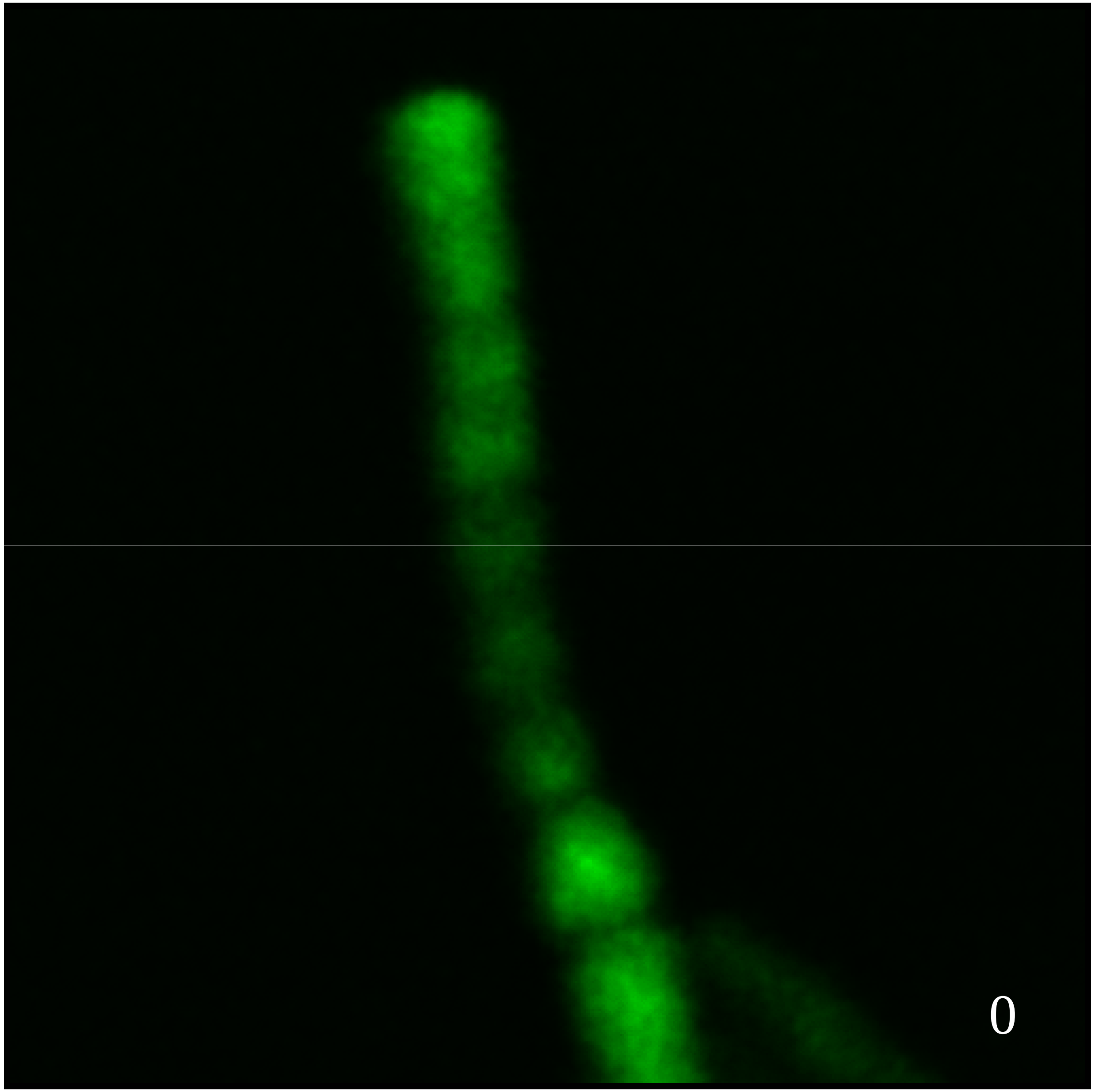
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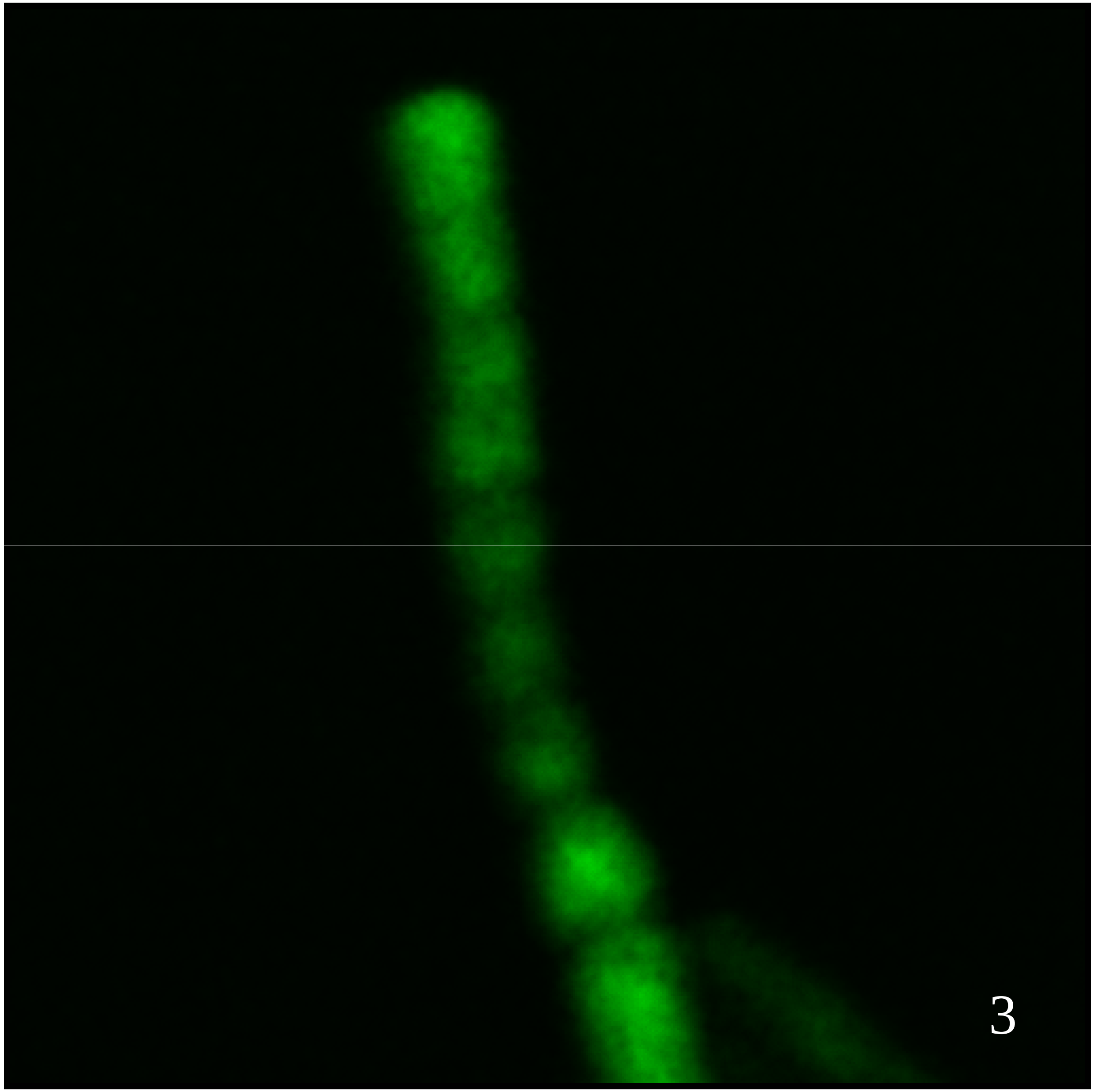
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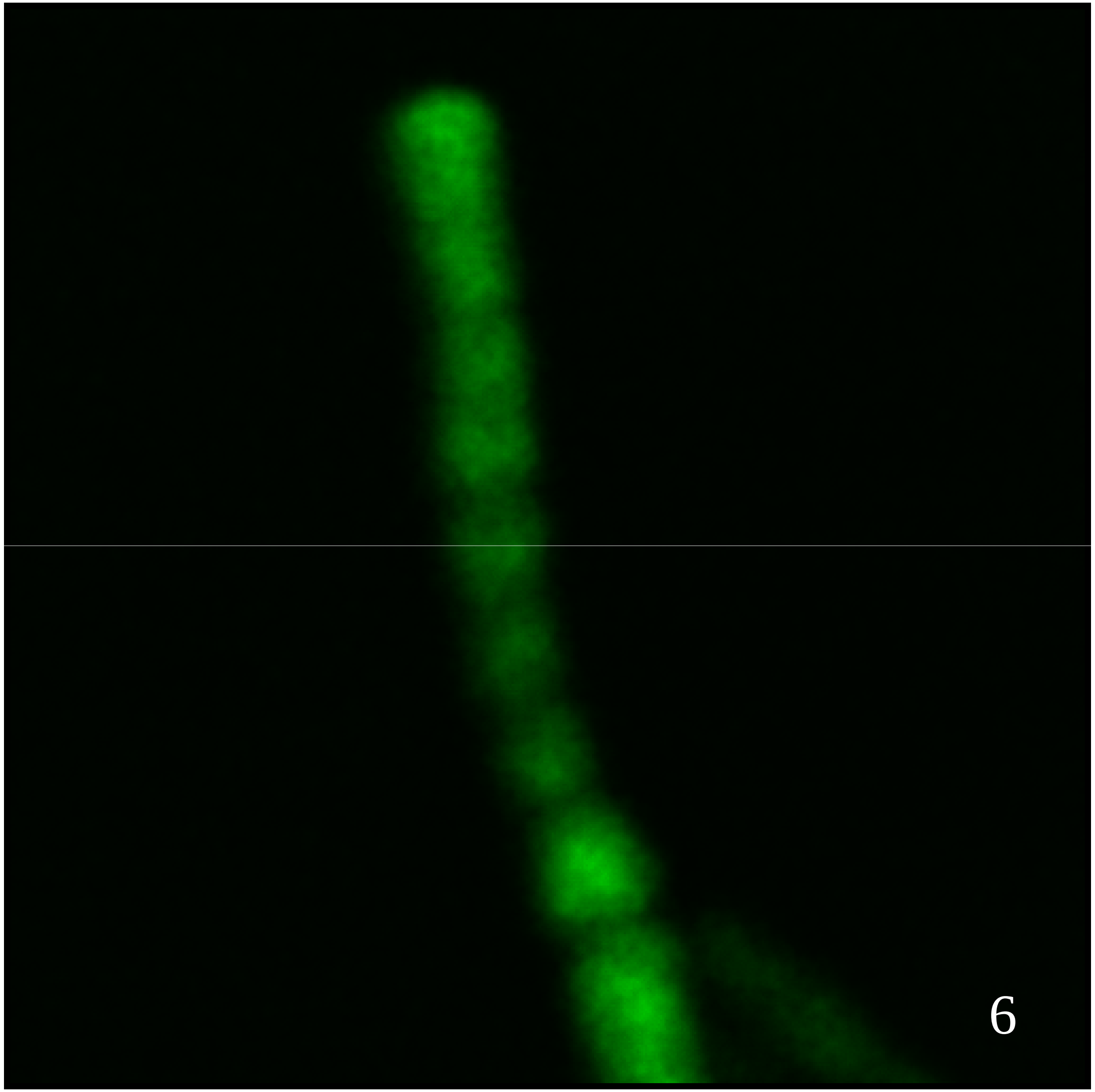
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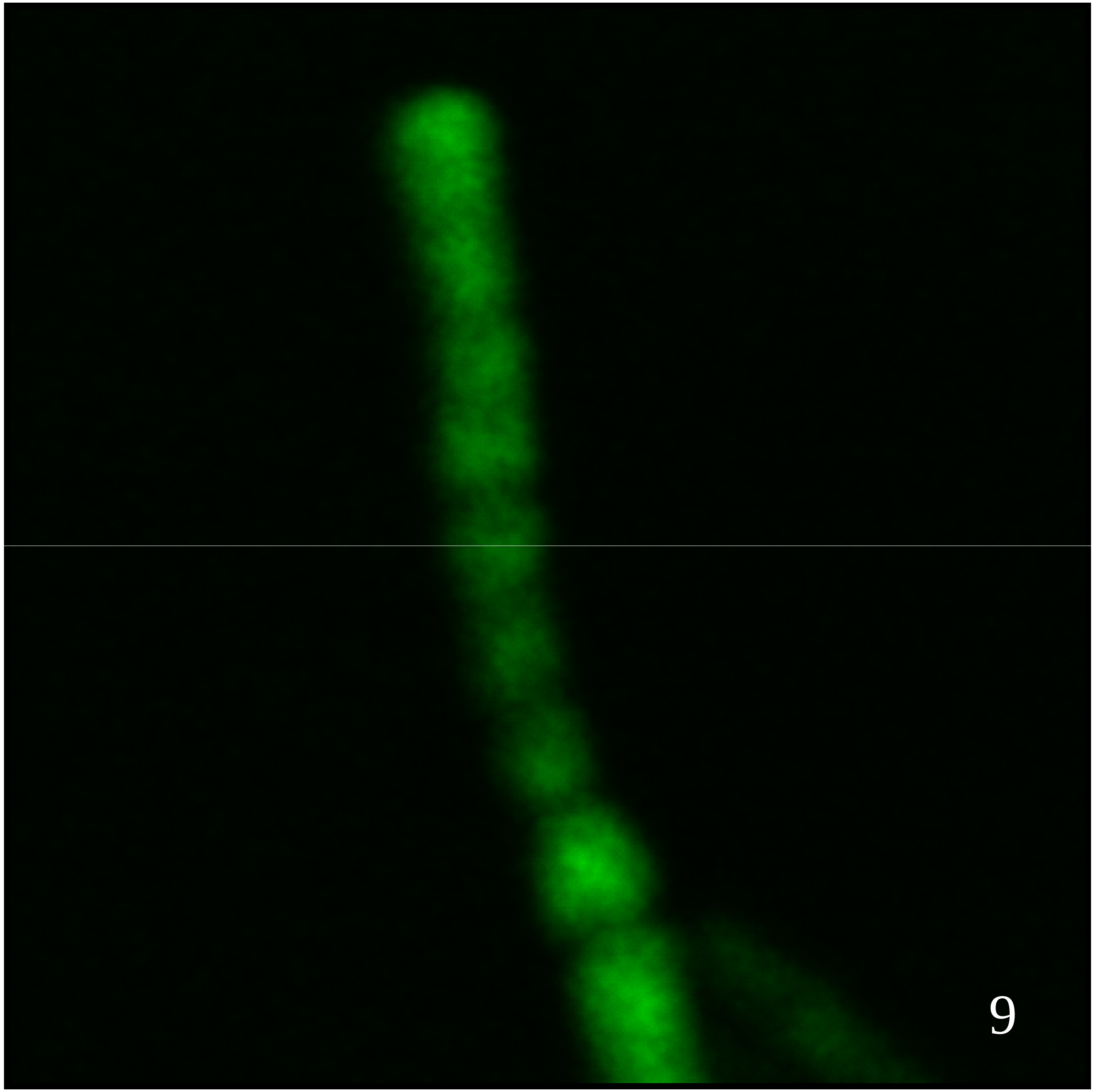
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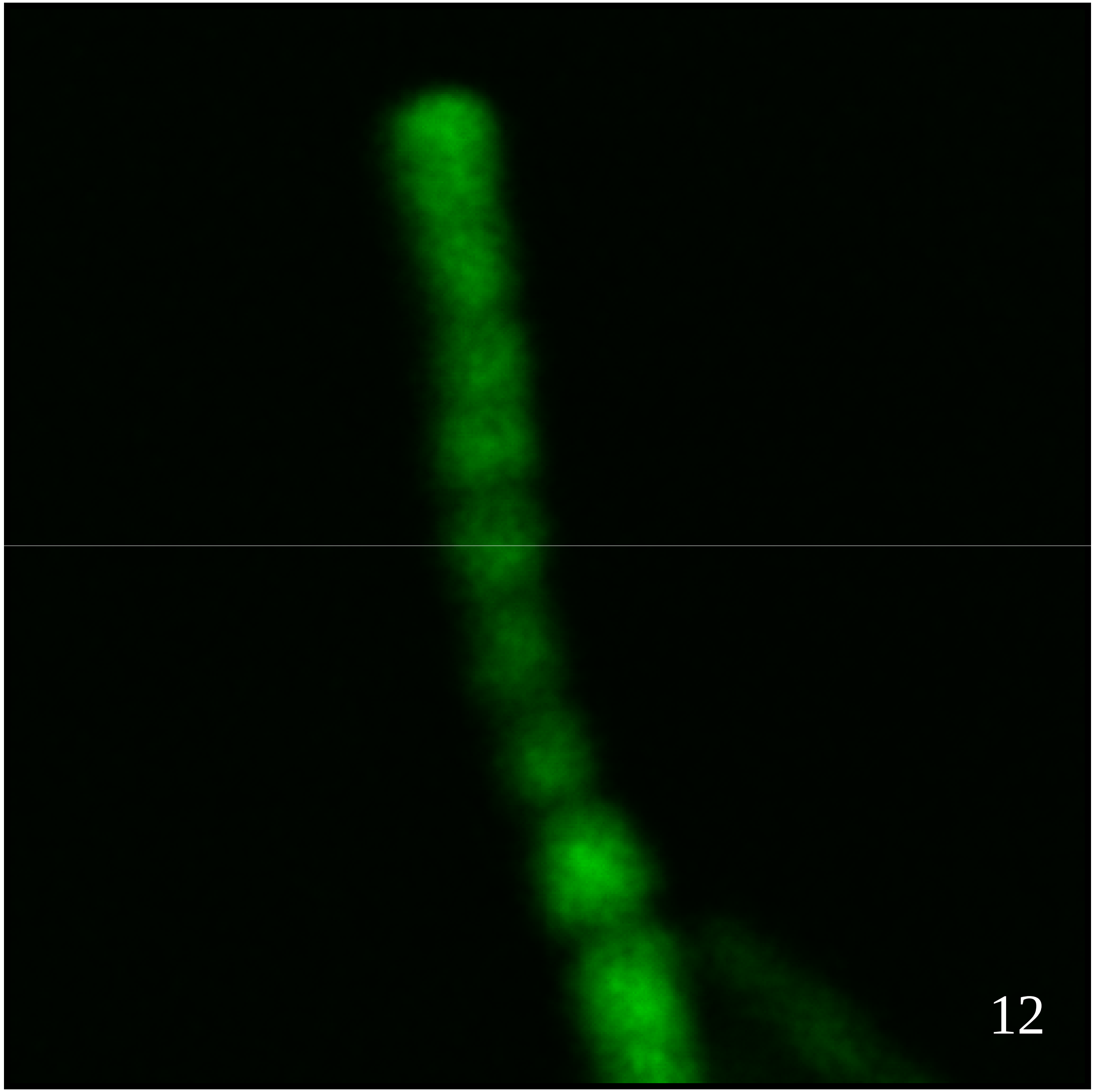
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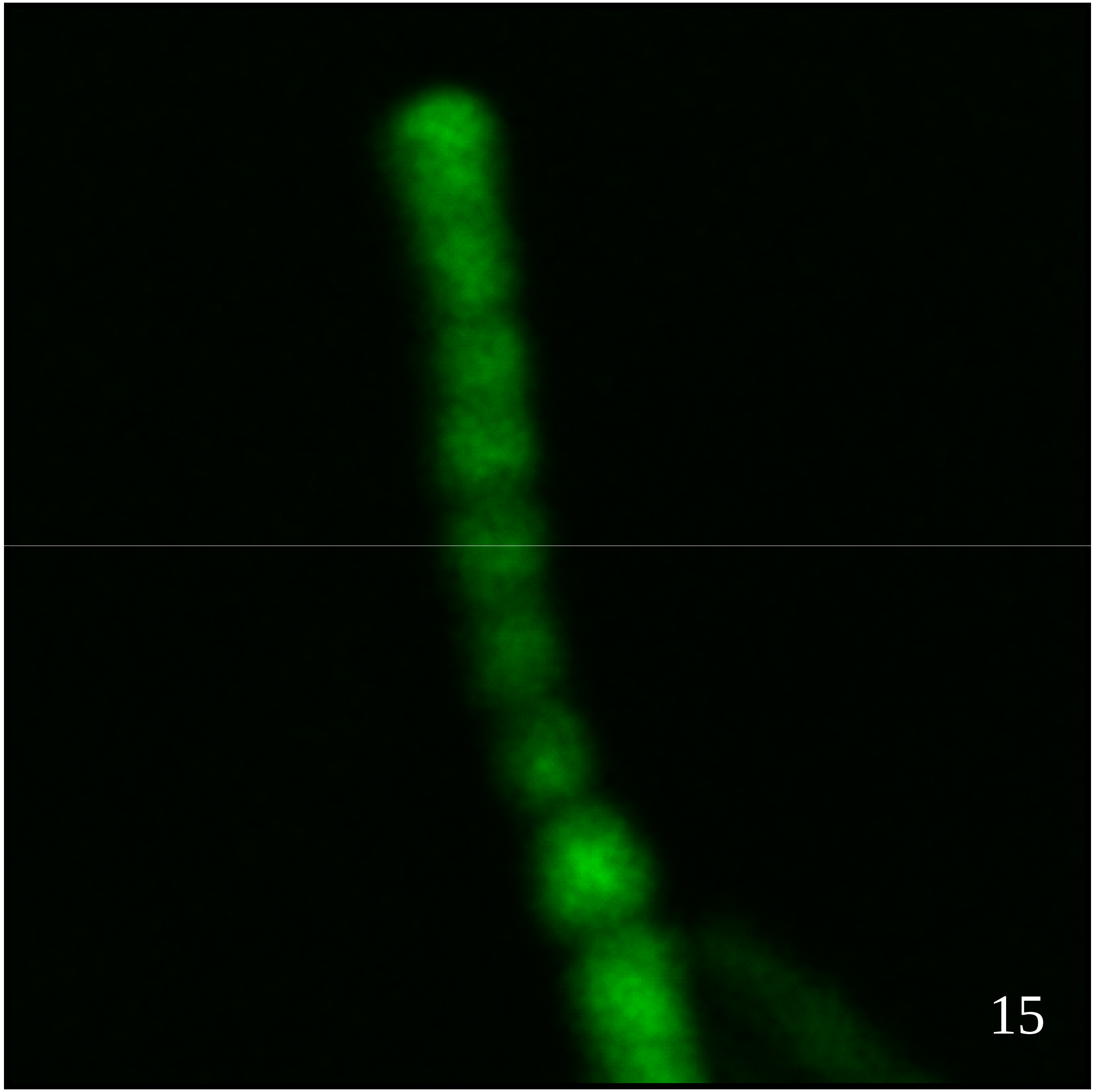
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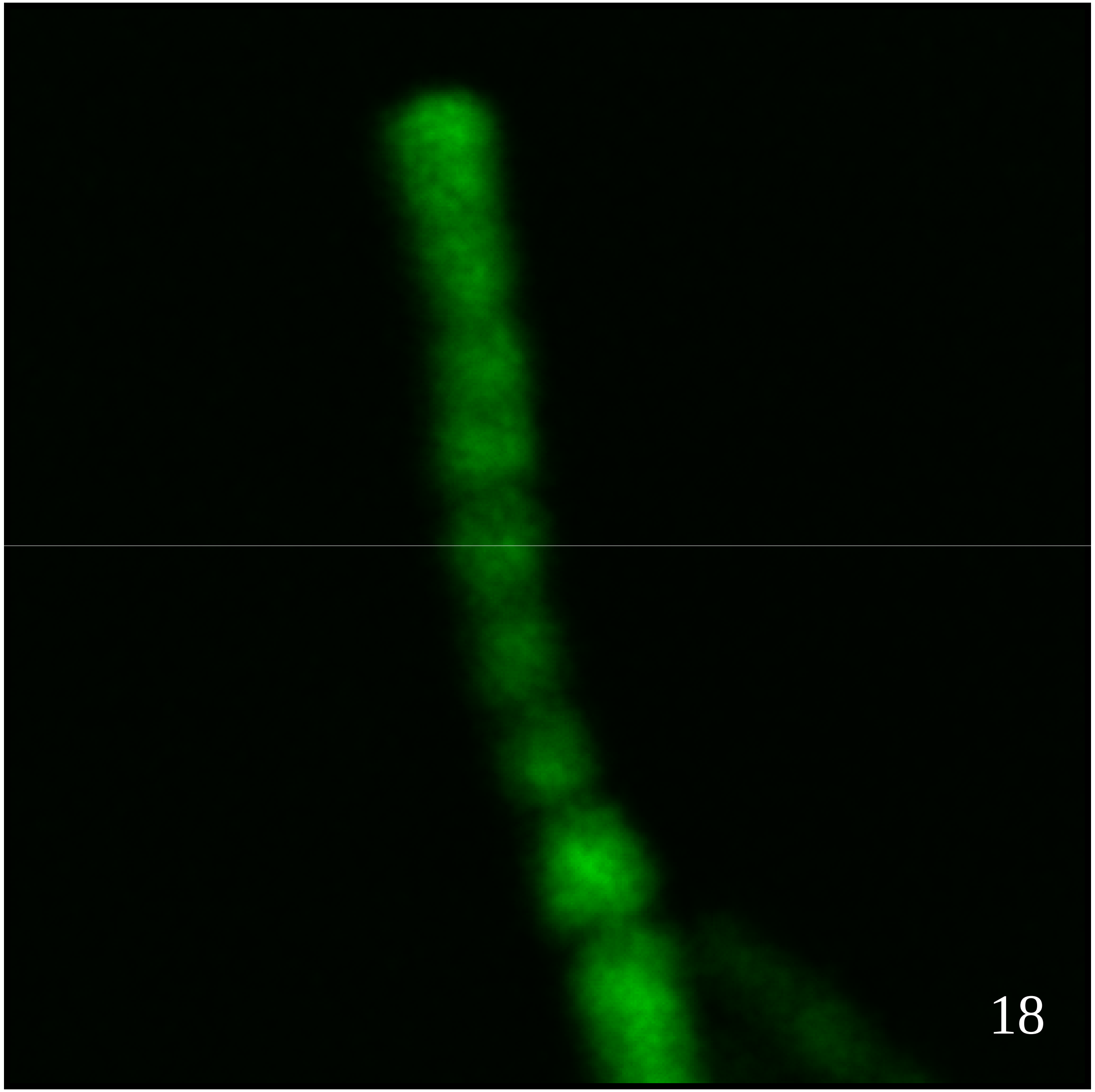
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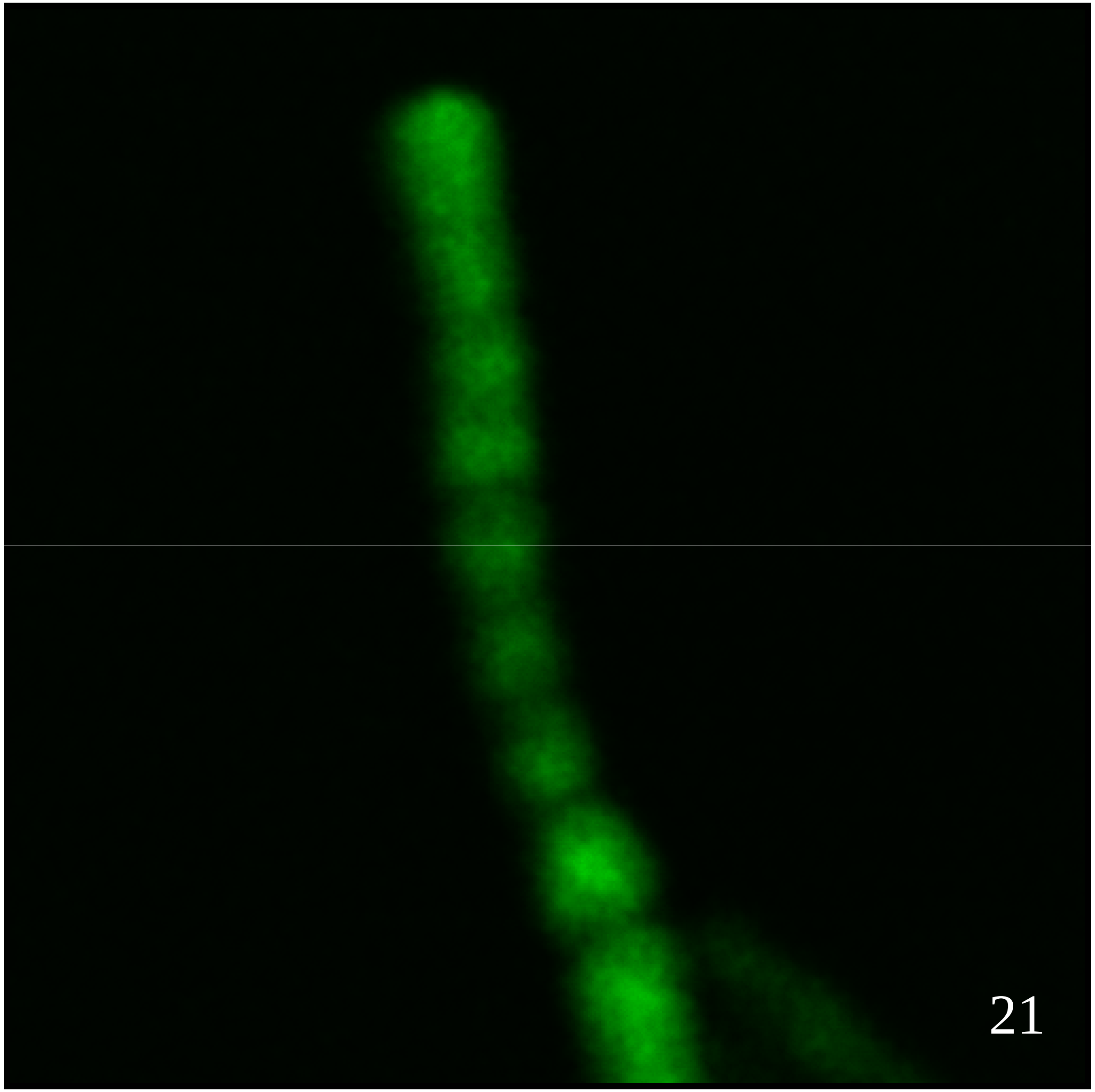
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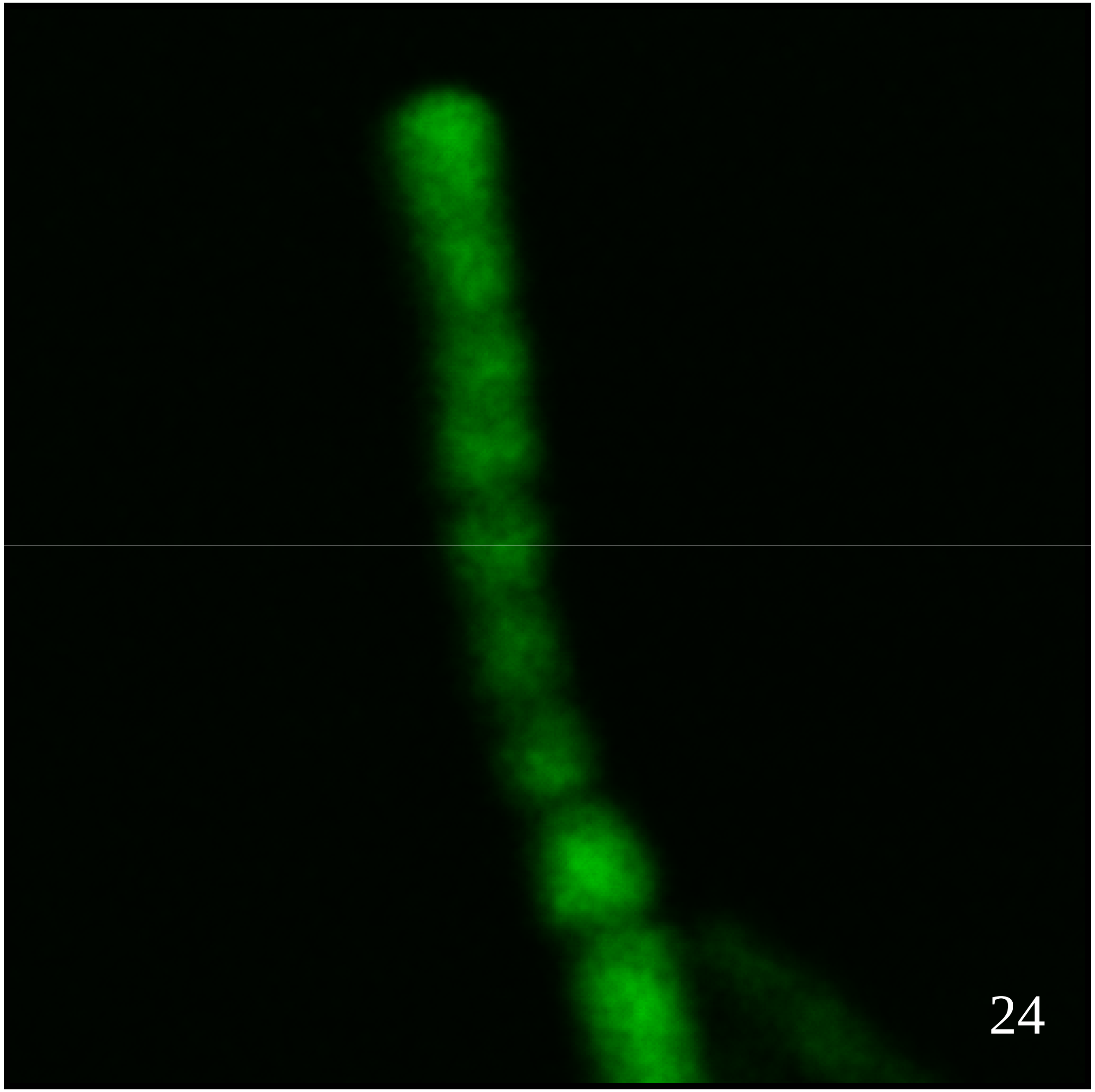
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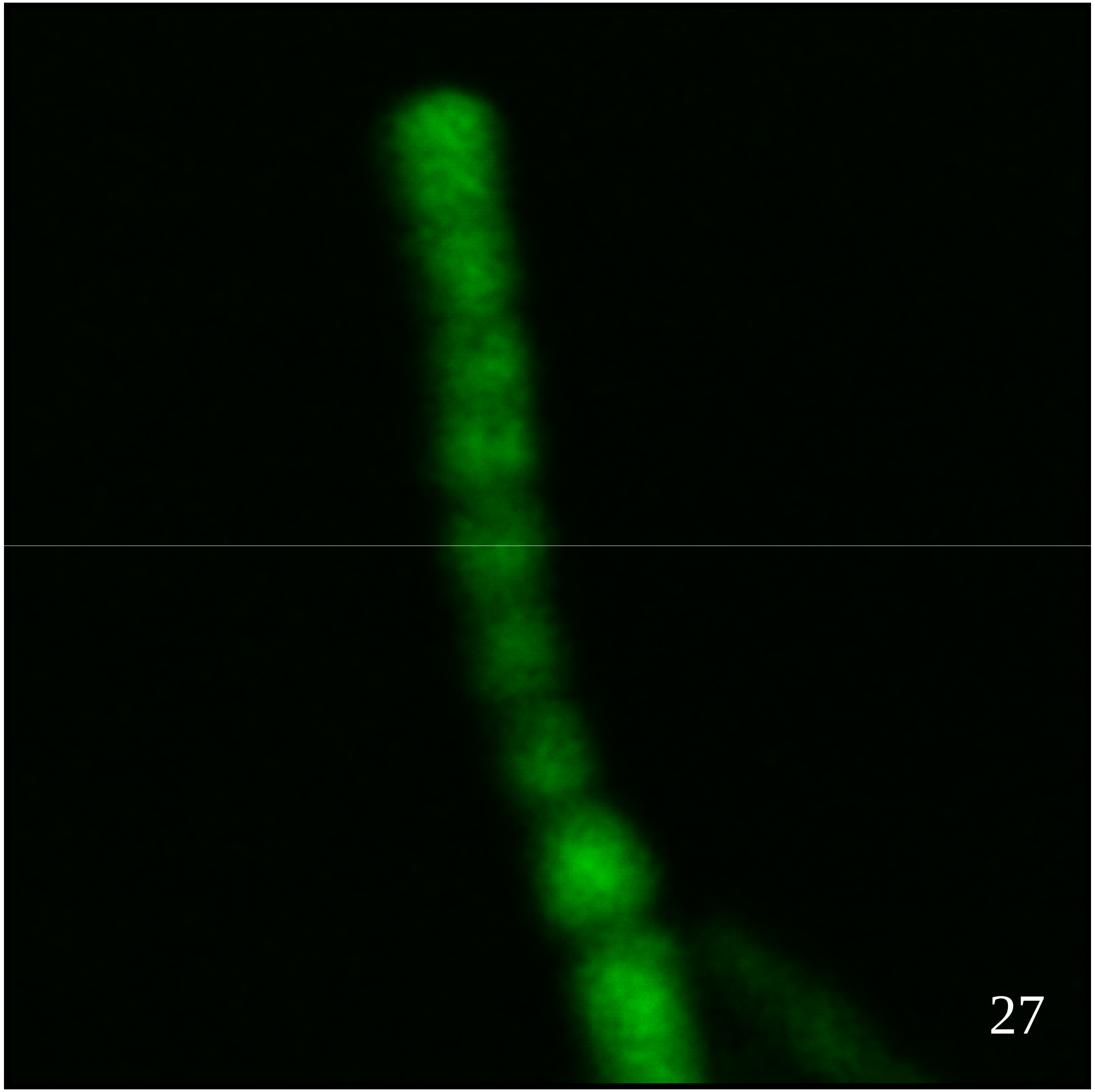
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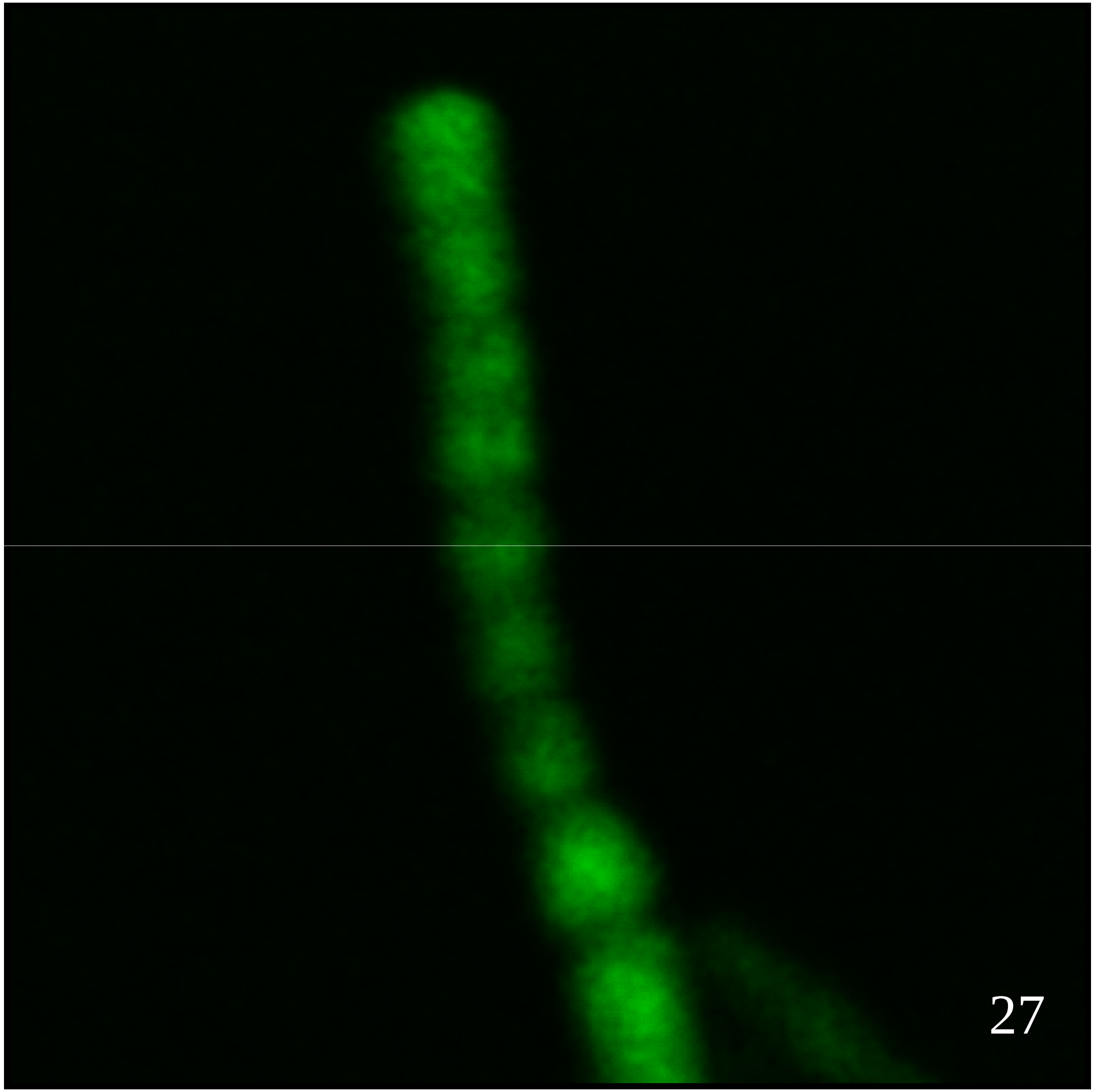
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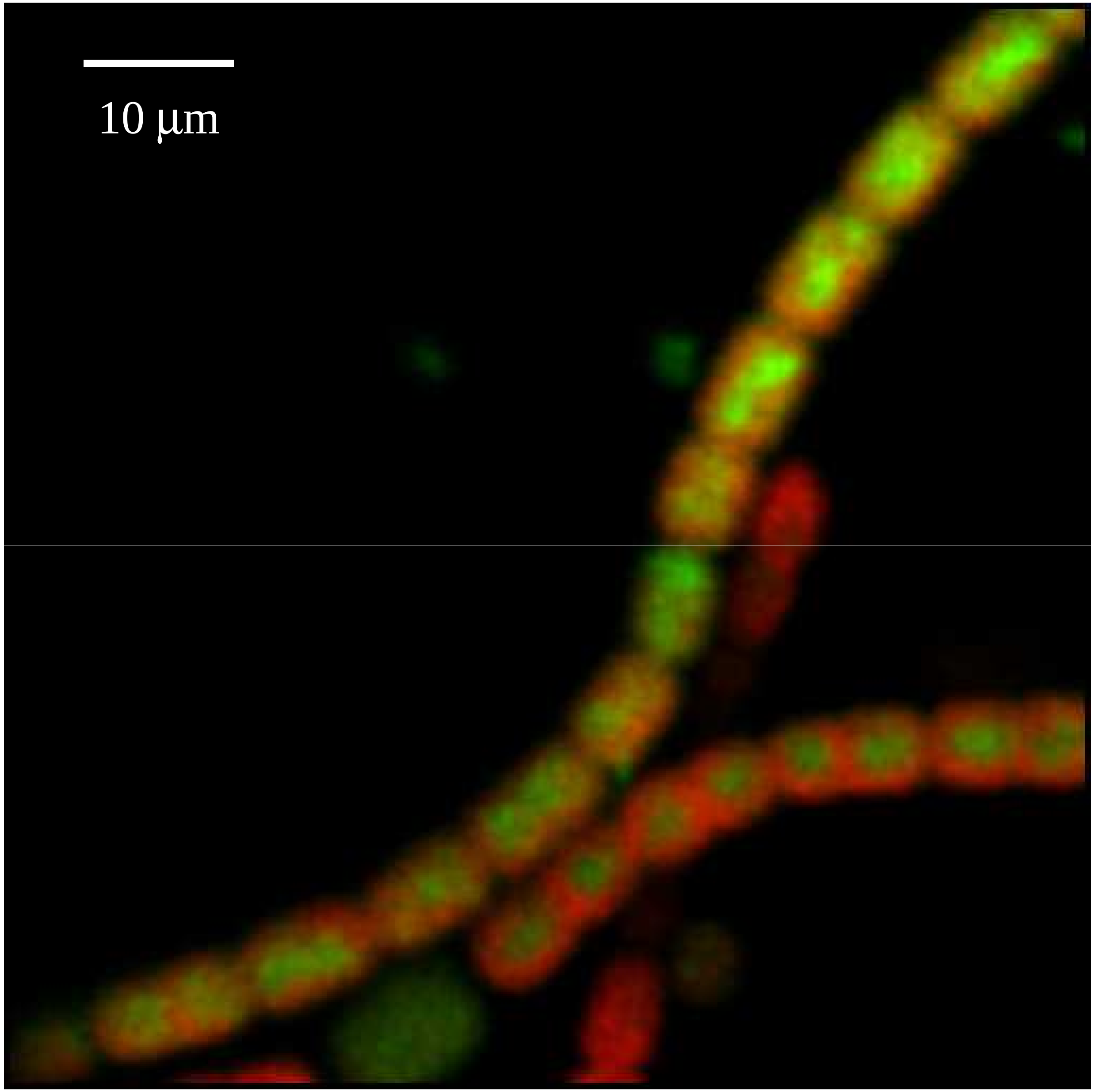
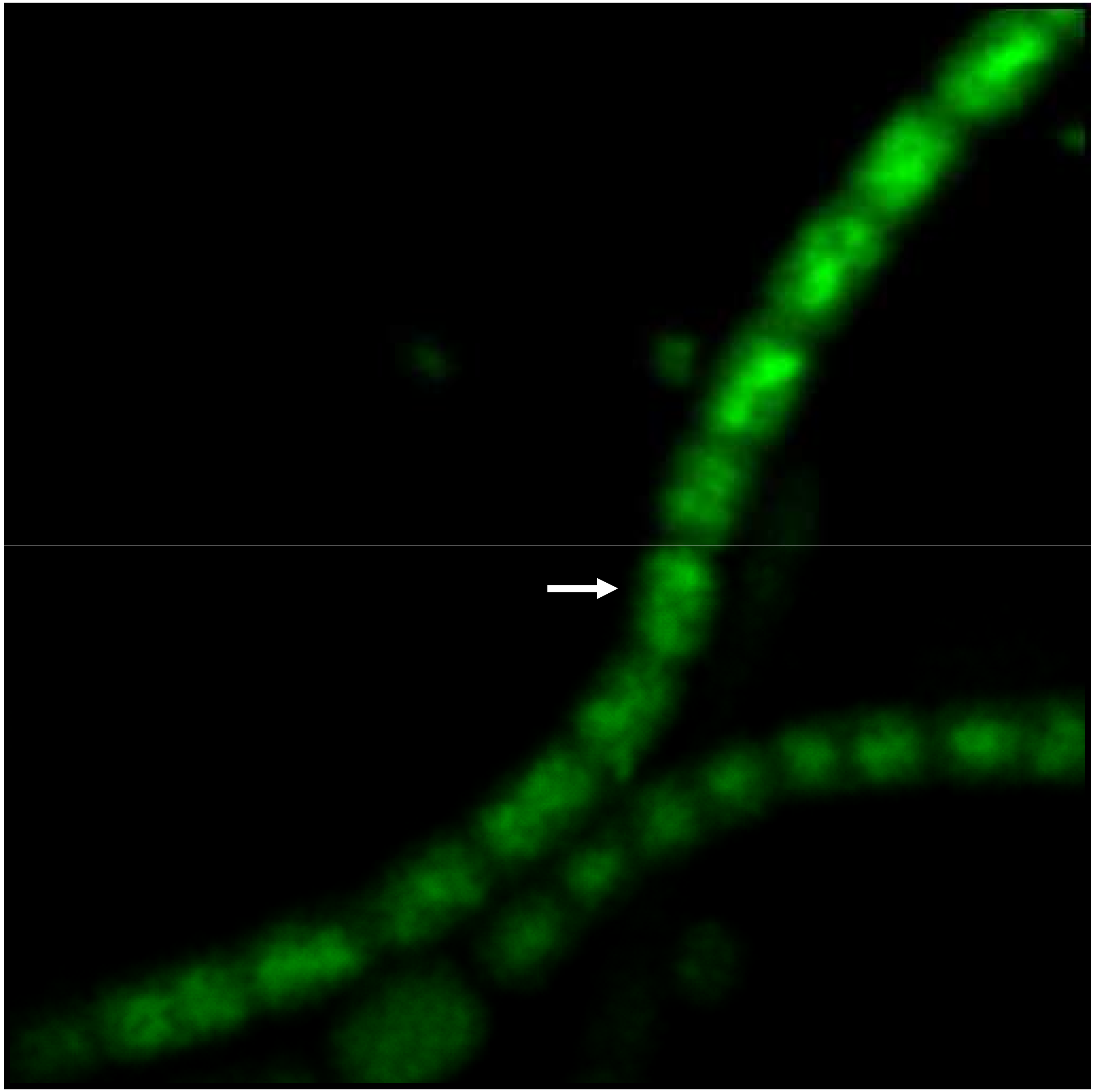
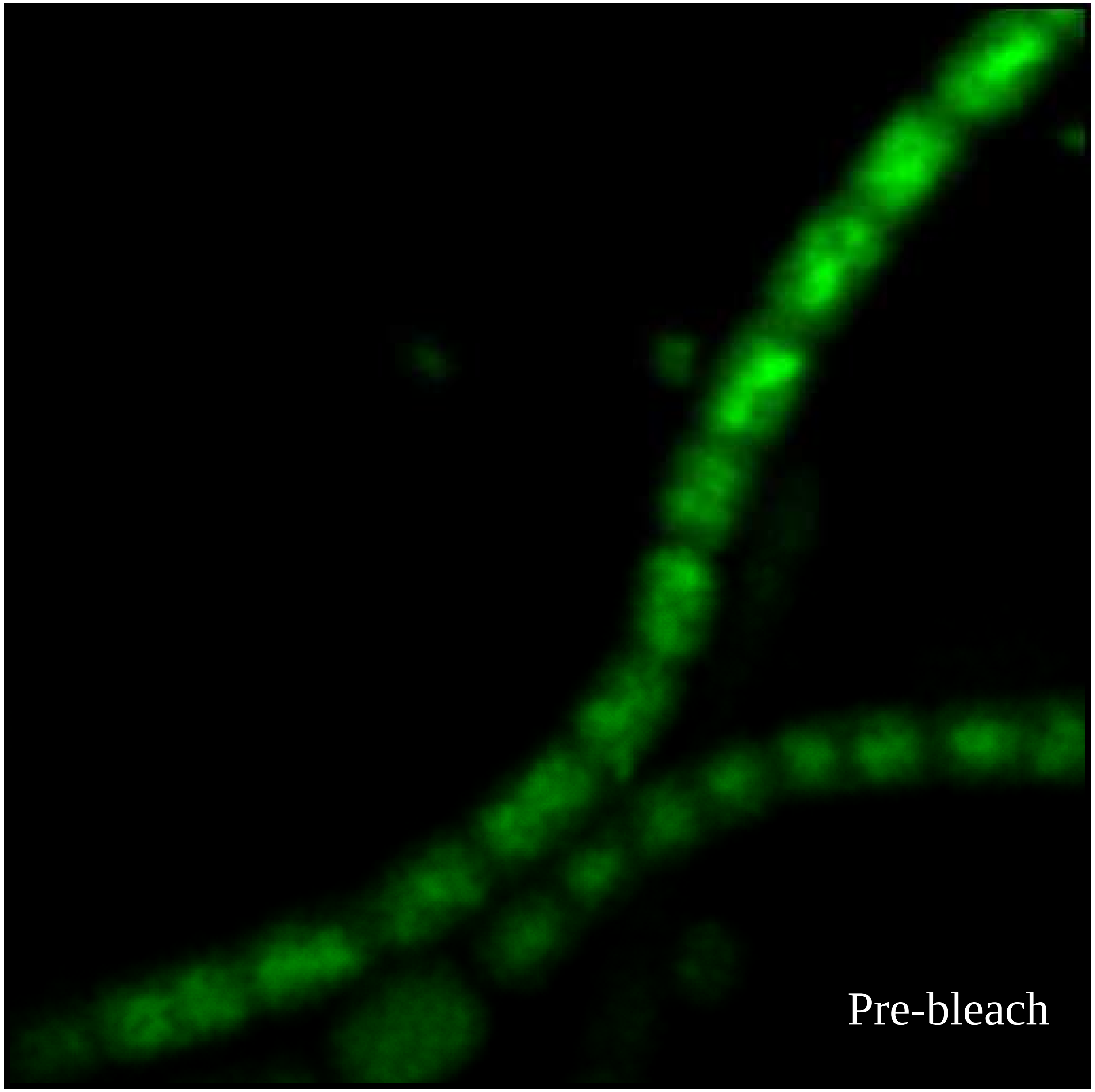
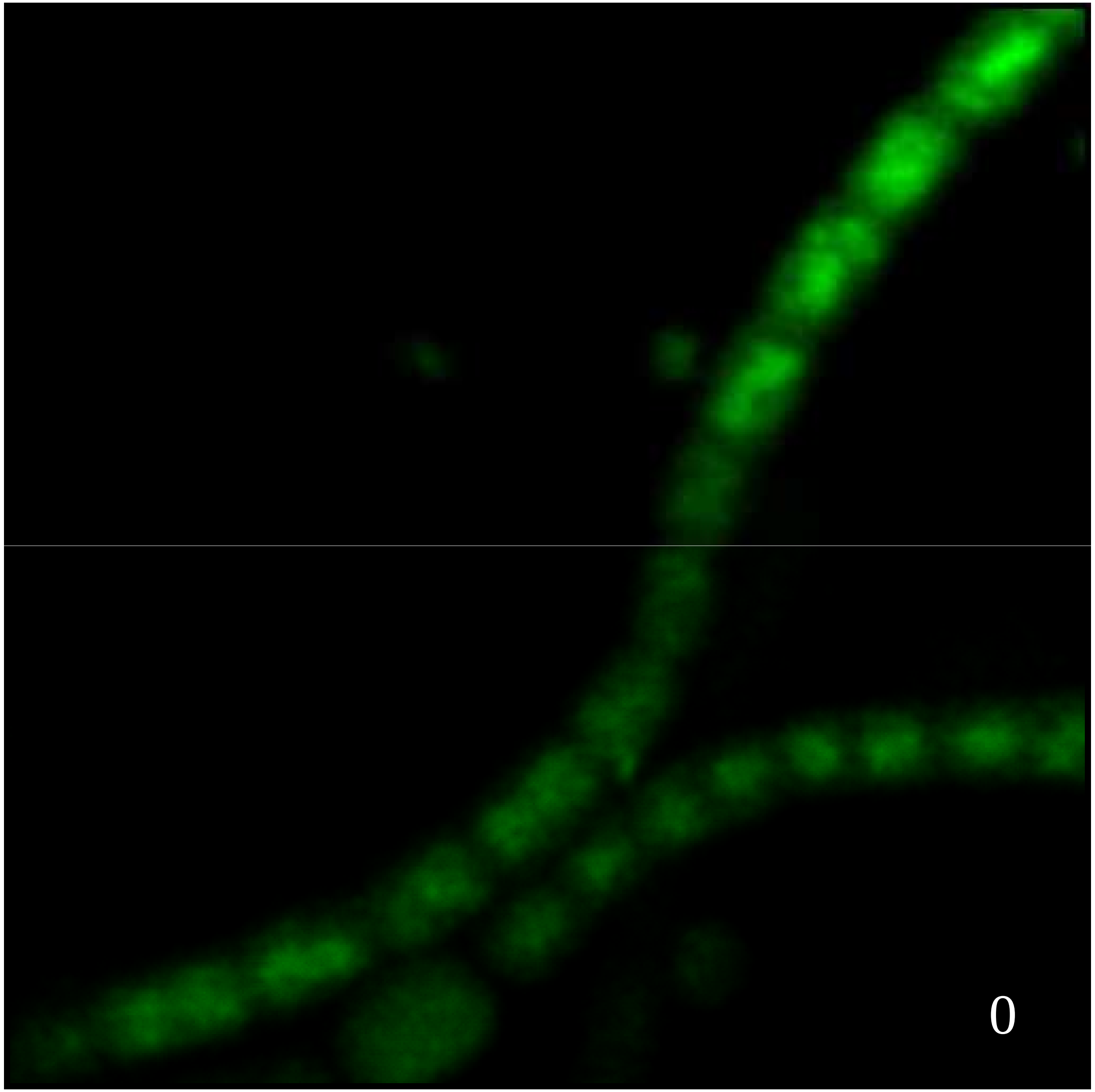
Calcein FRAP in *Anabaena cylindrica* (N_2-fixing)



Dye exchange between vegetative cells




Pre-bleach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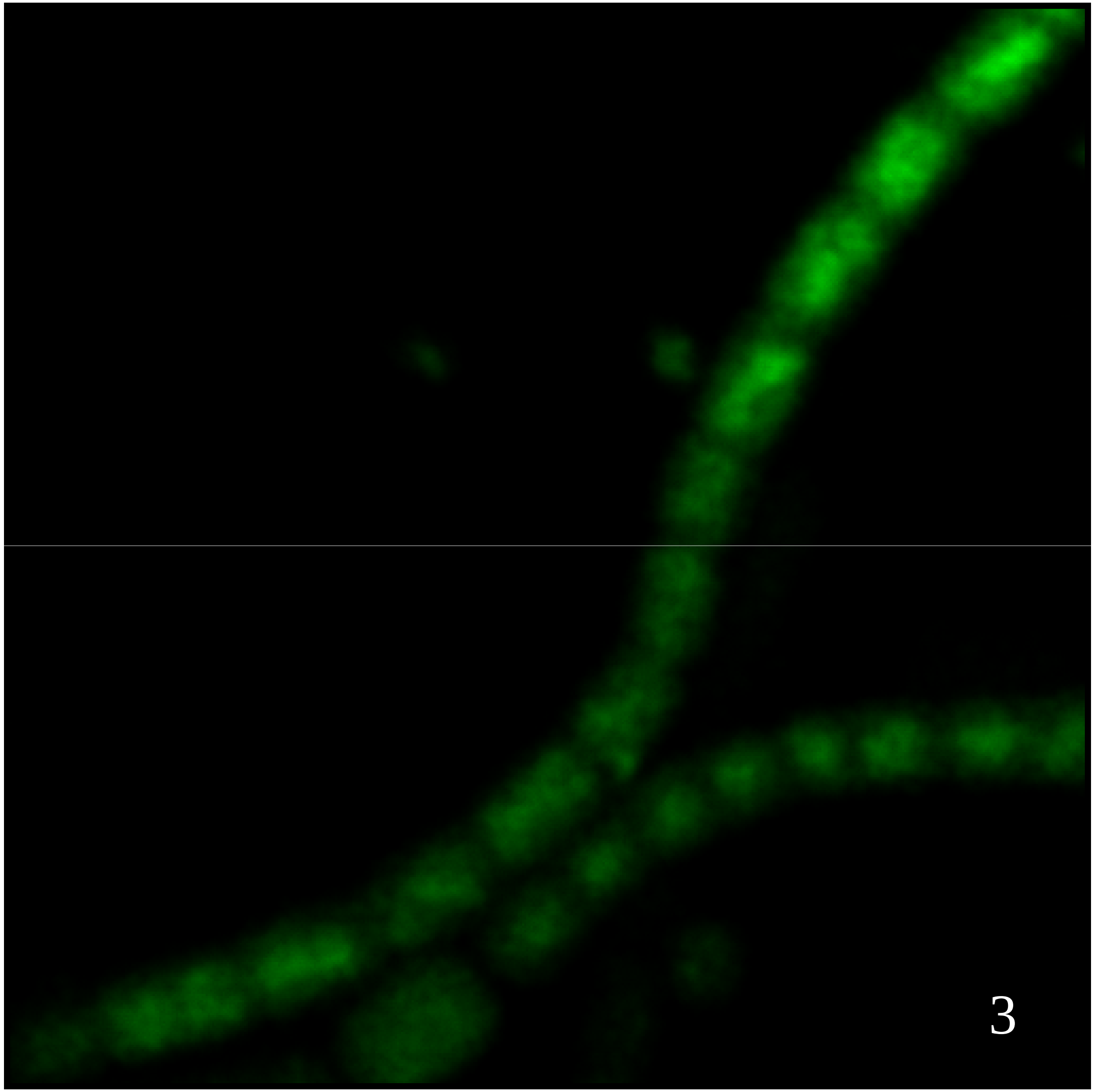
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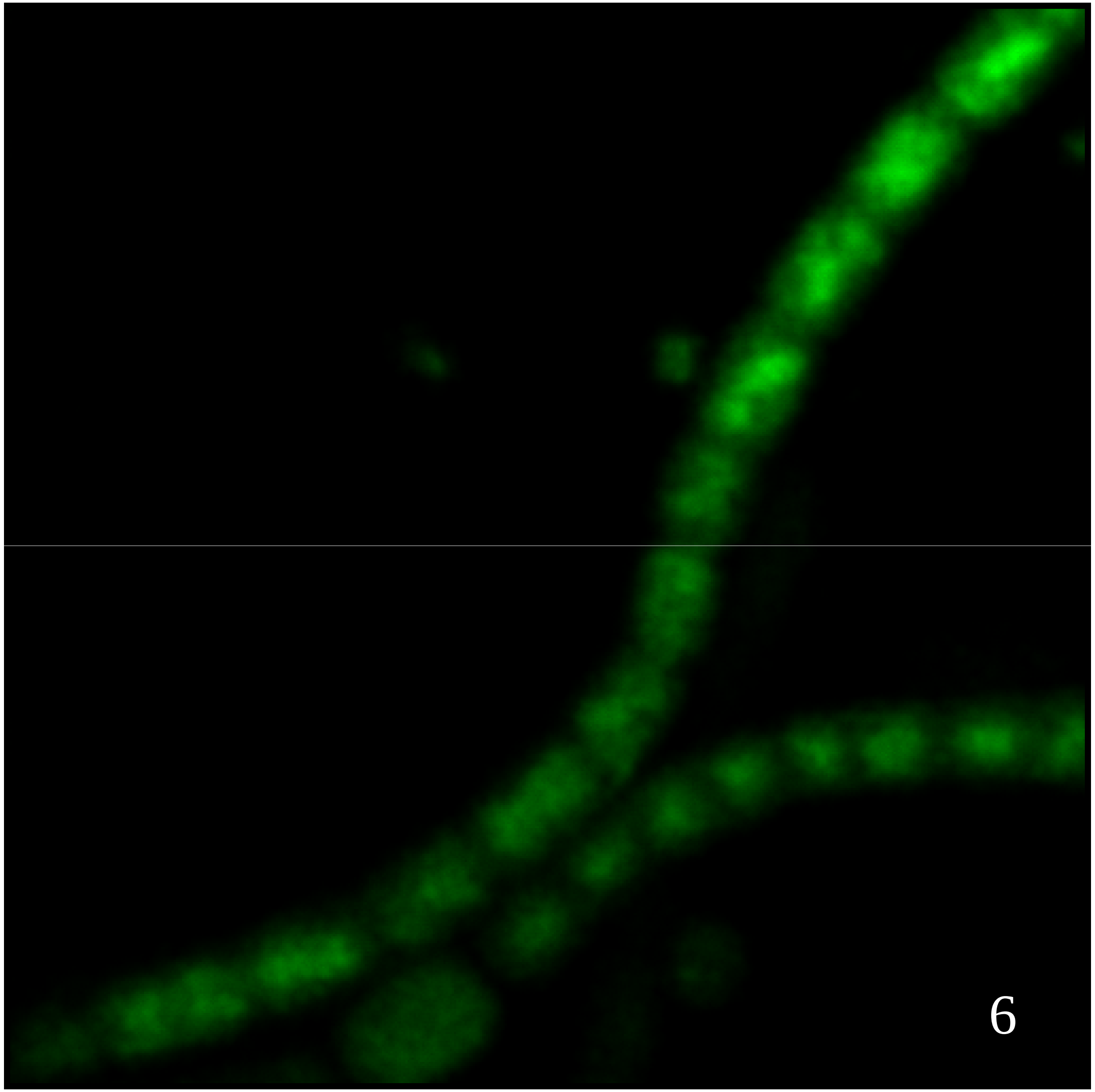
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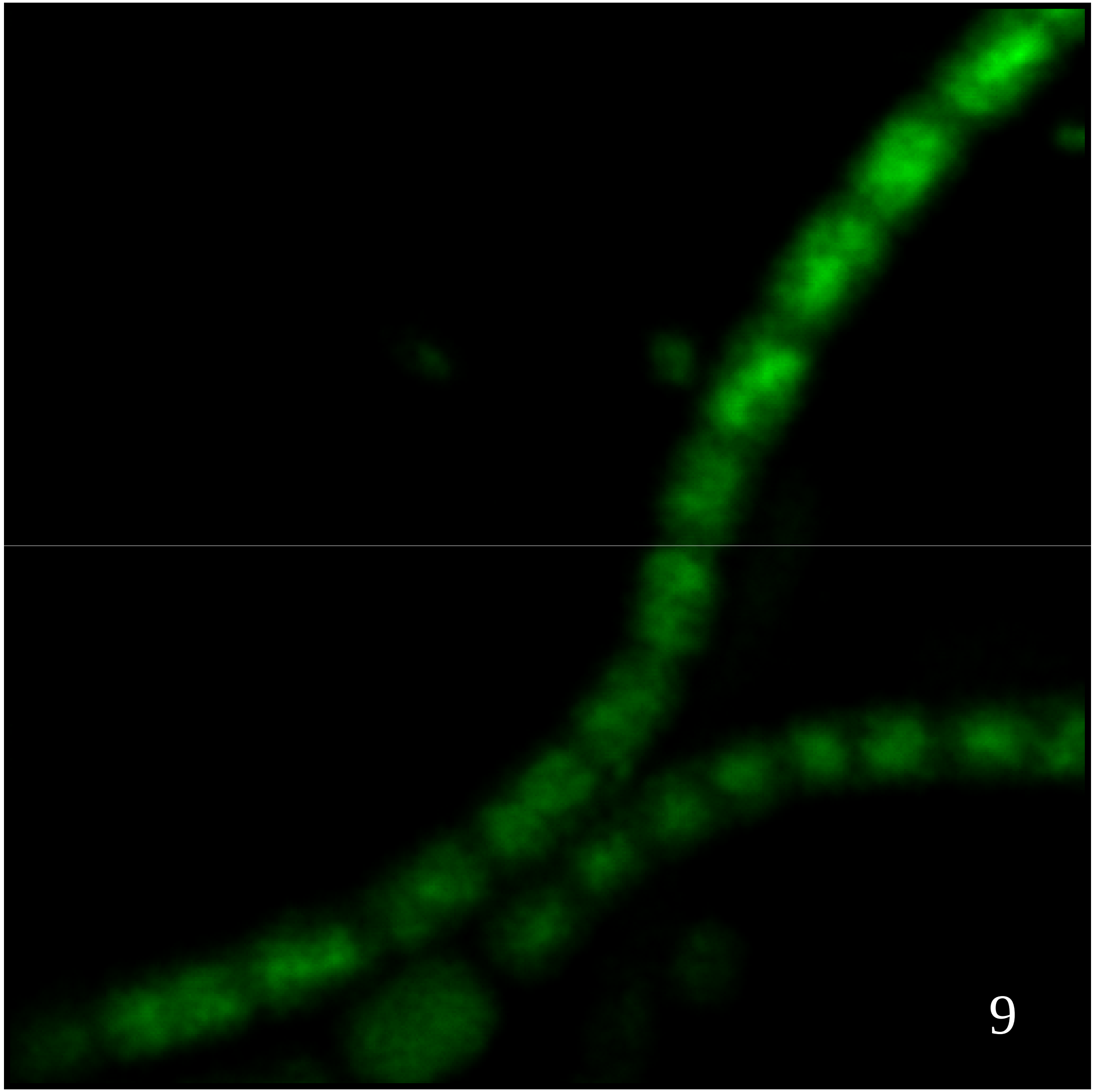
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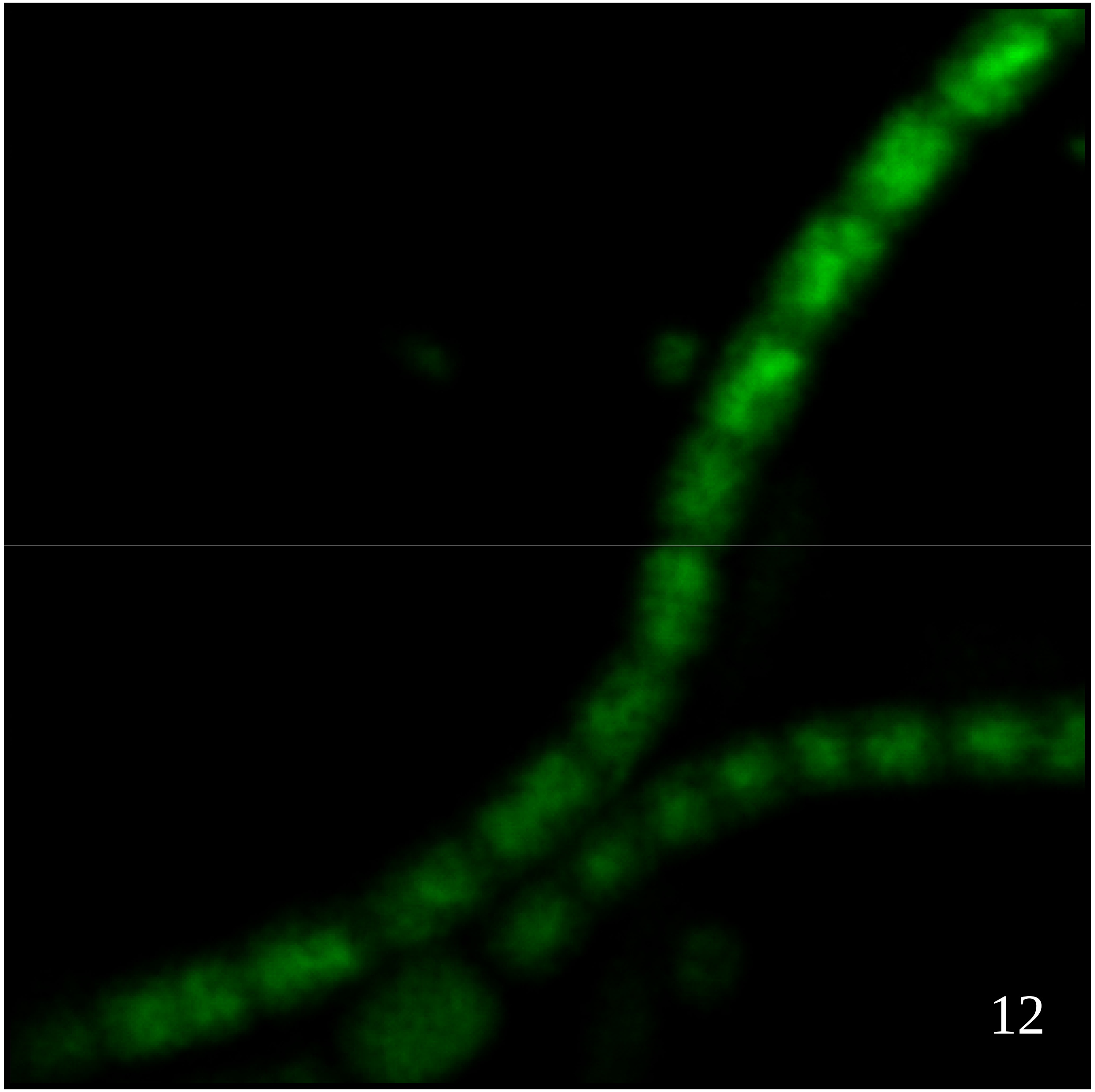
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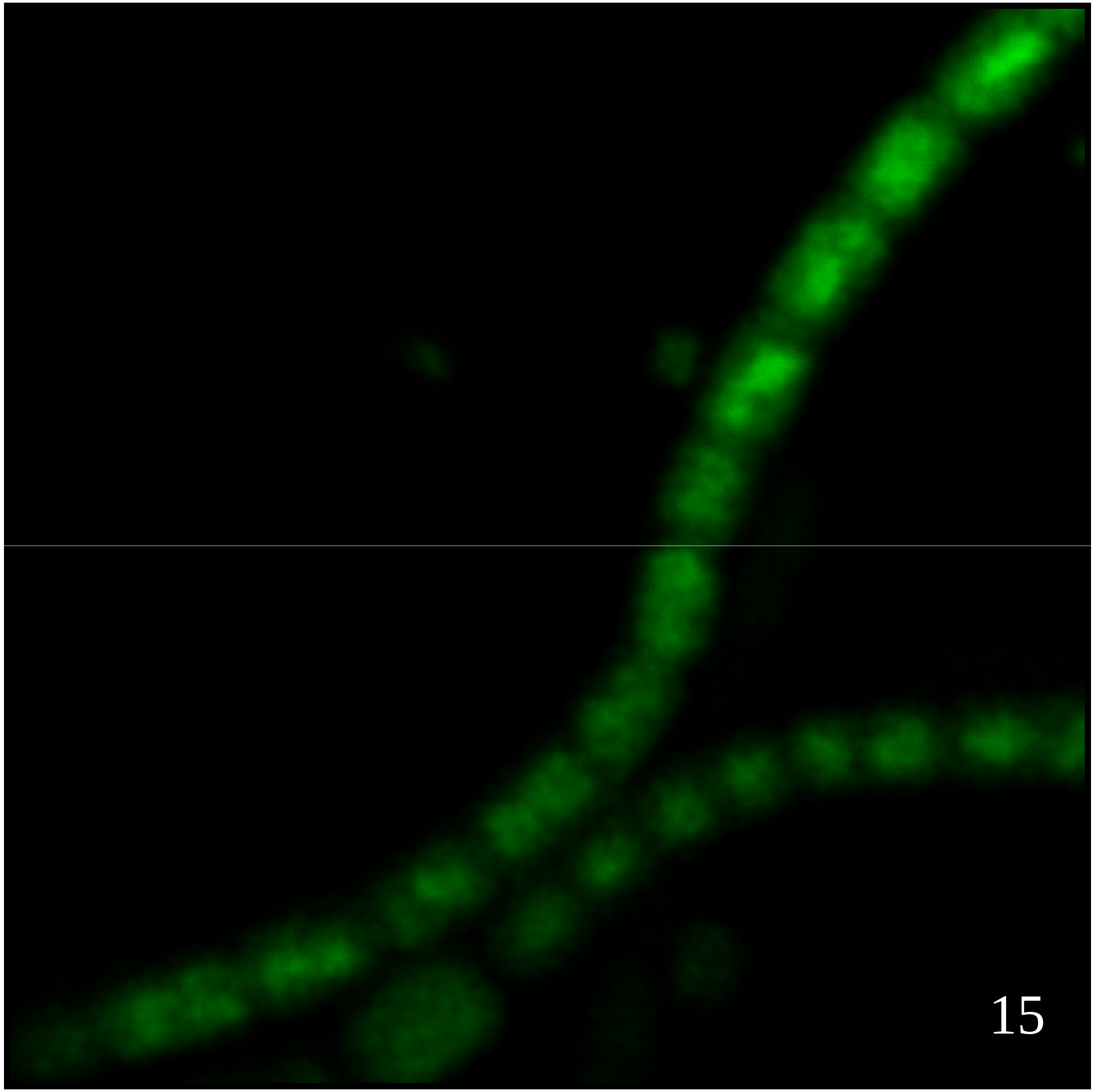
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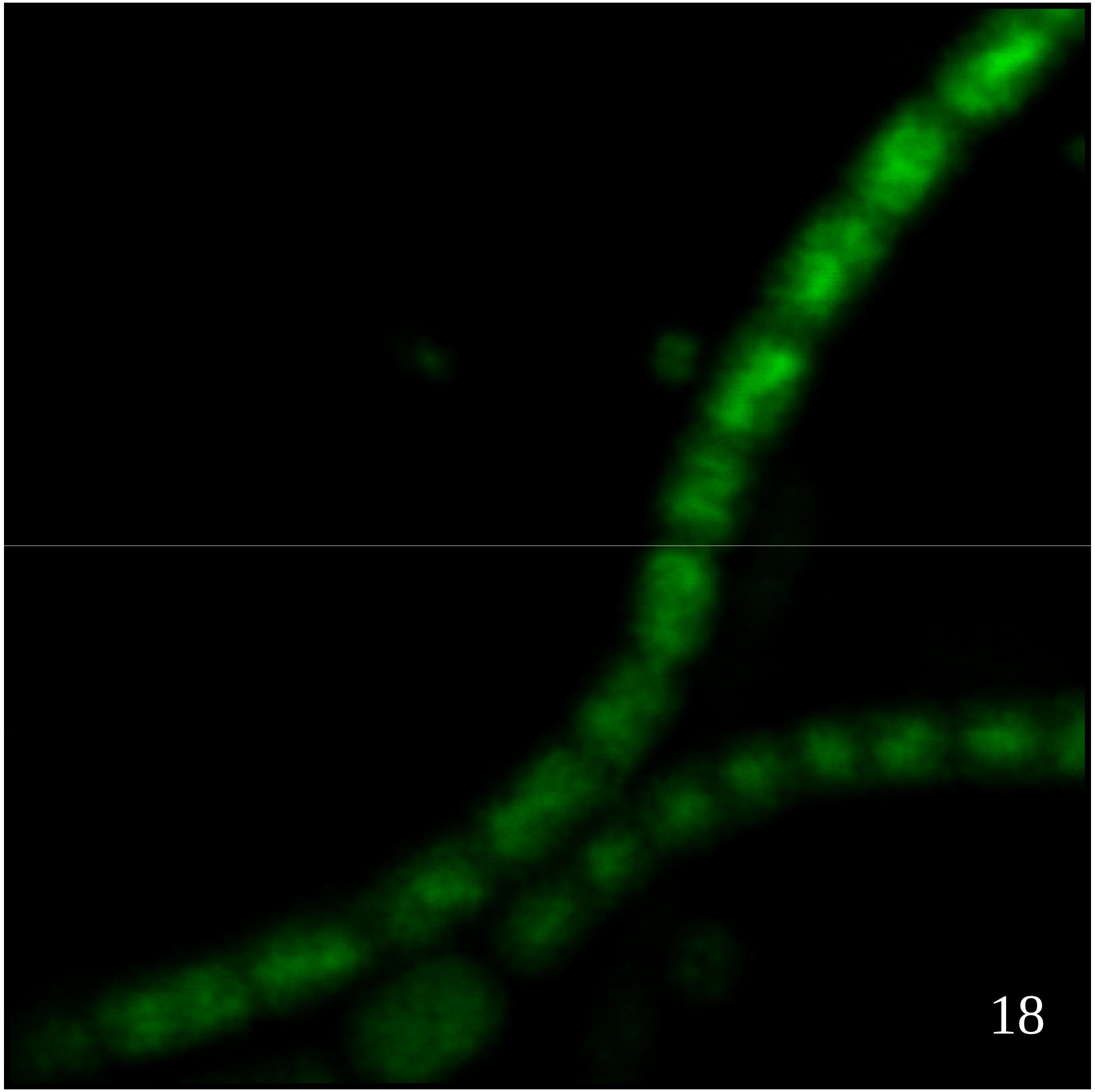
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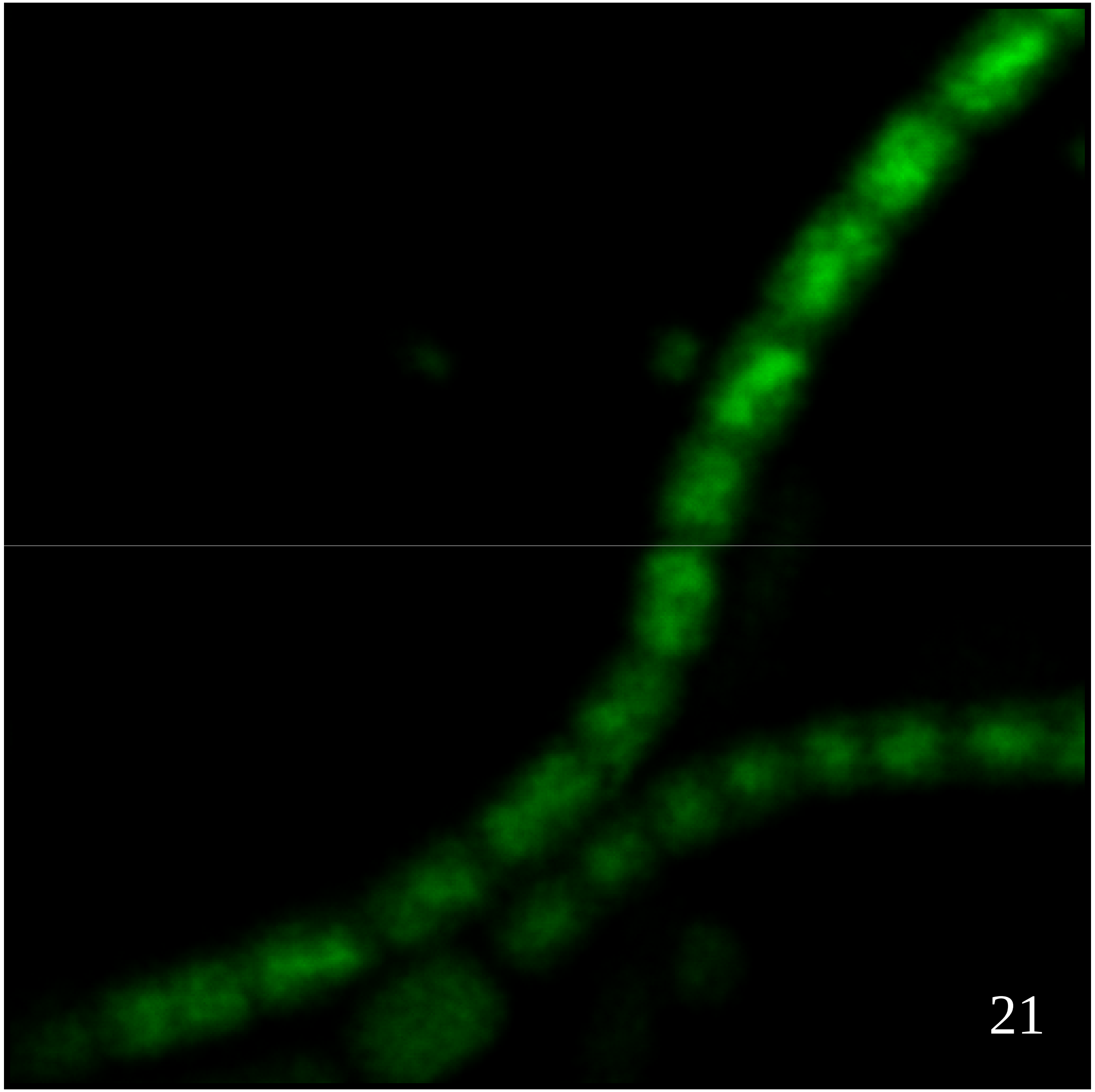
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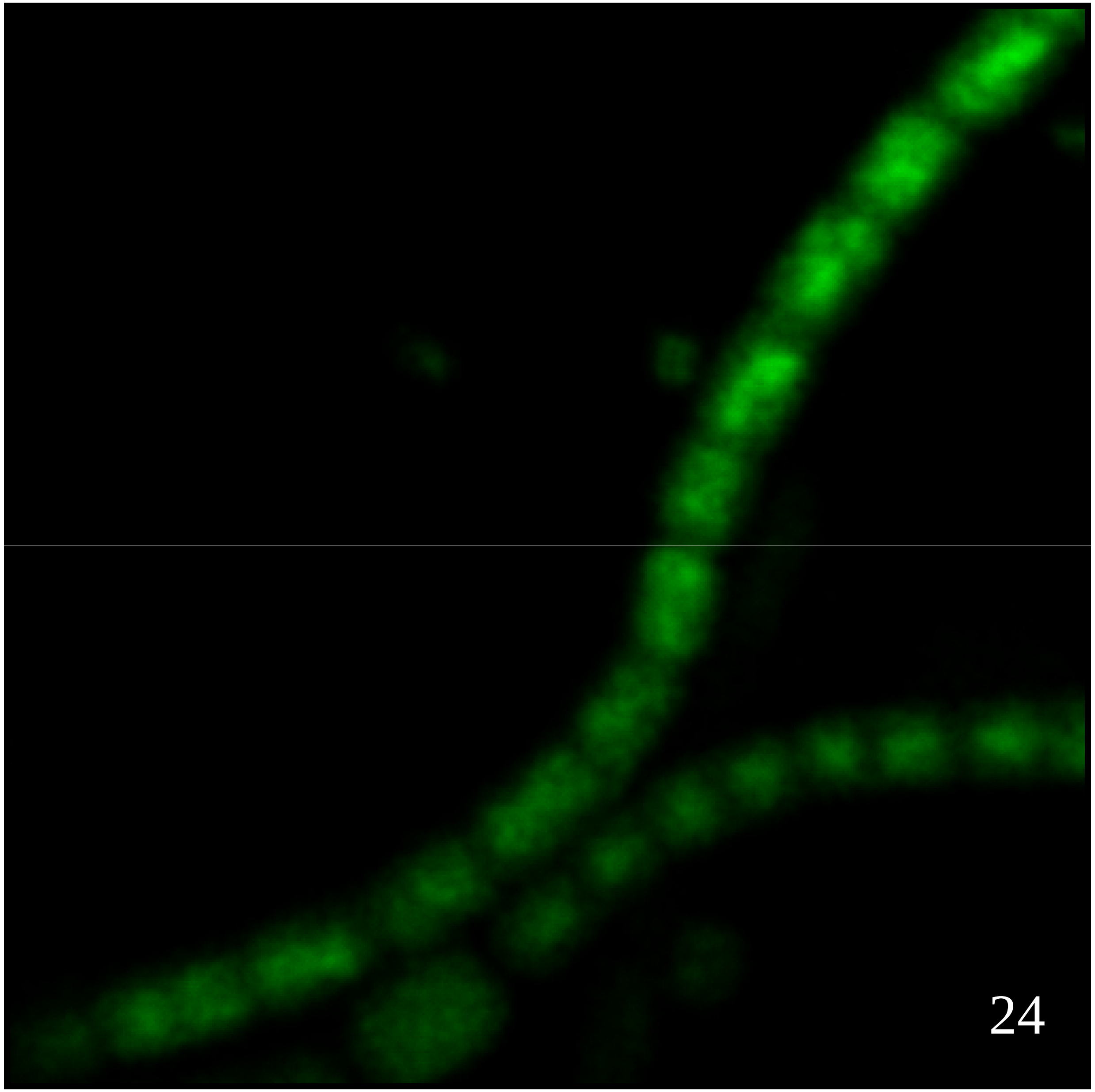
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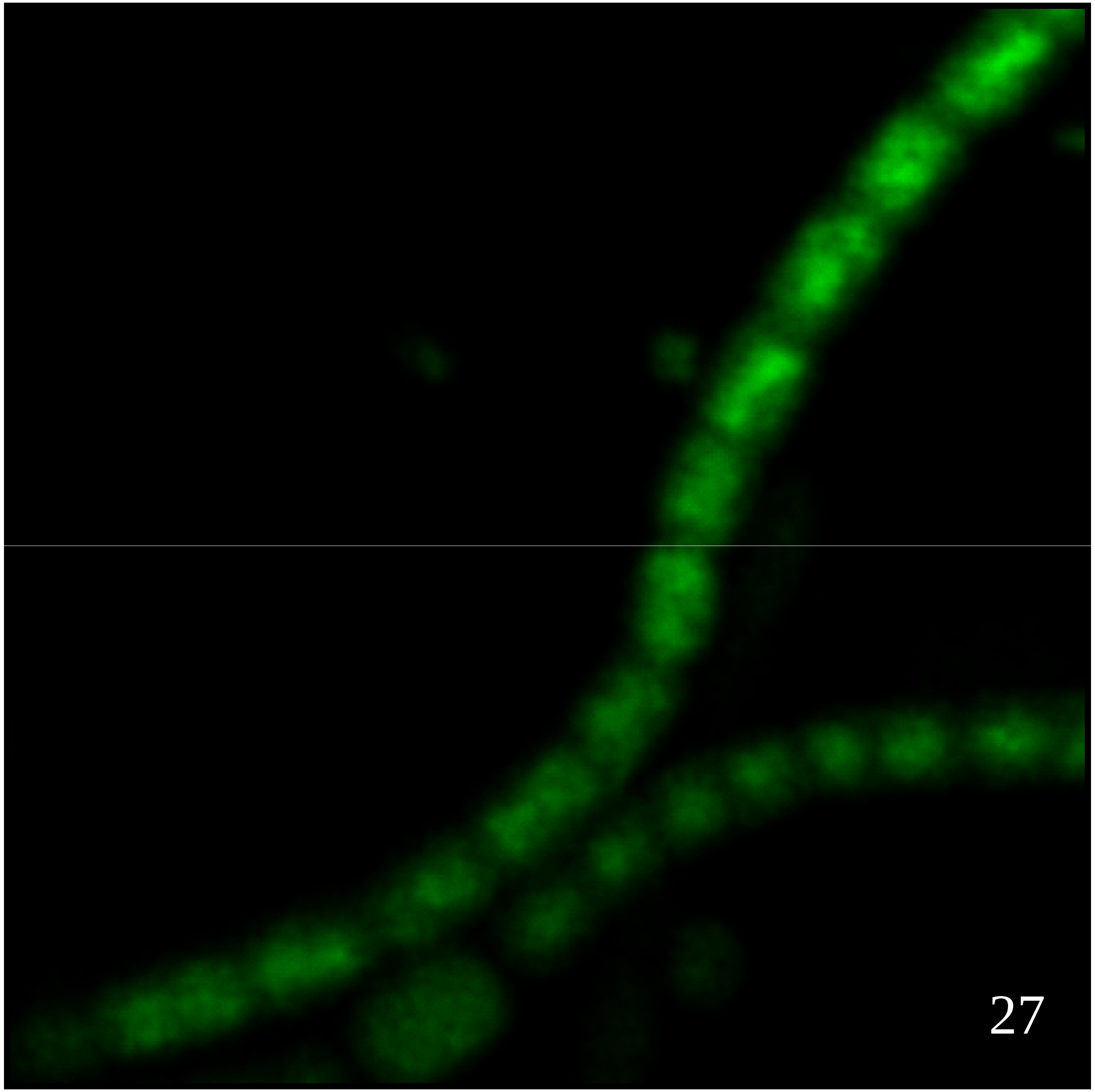
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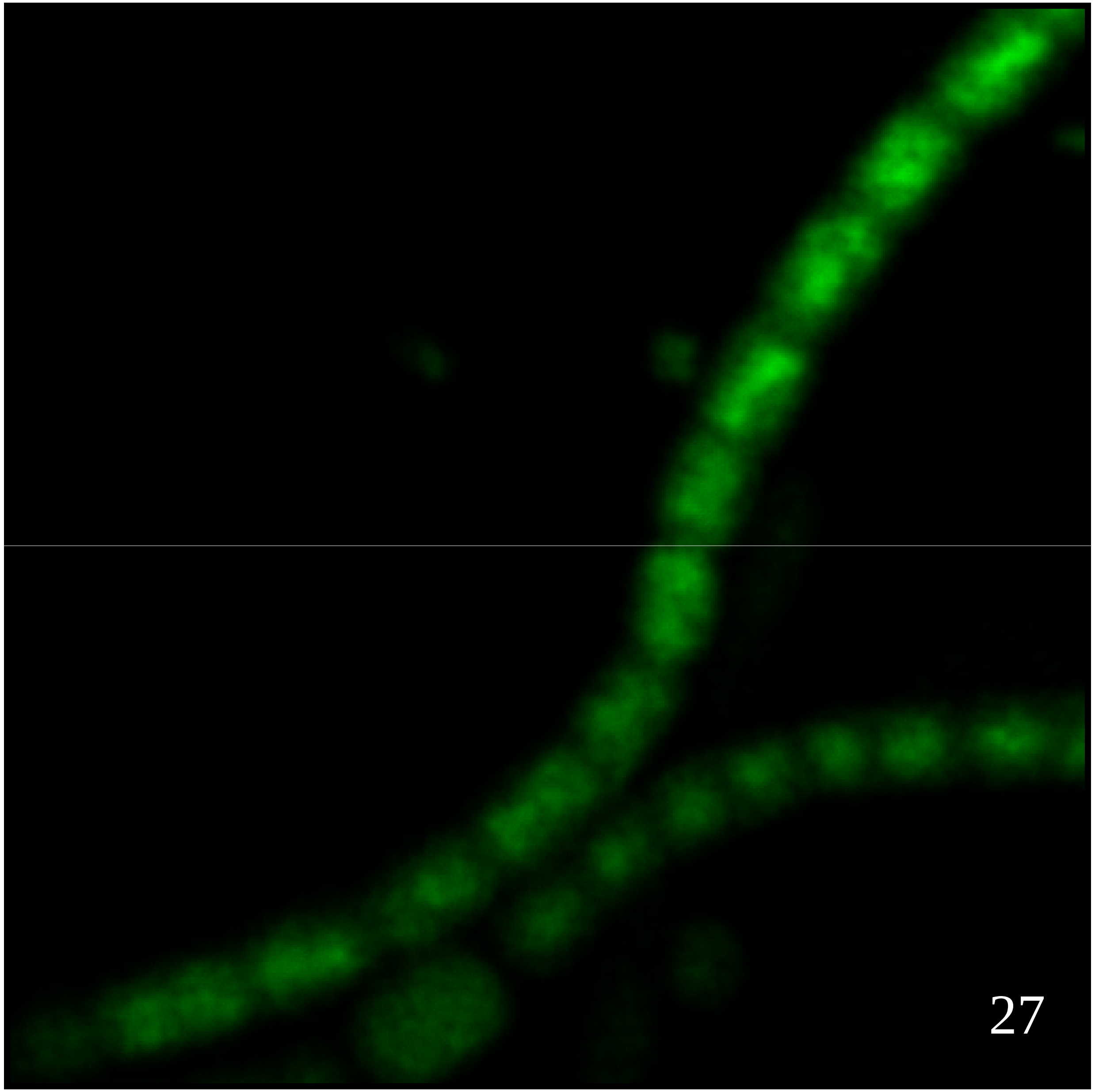
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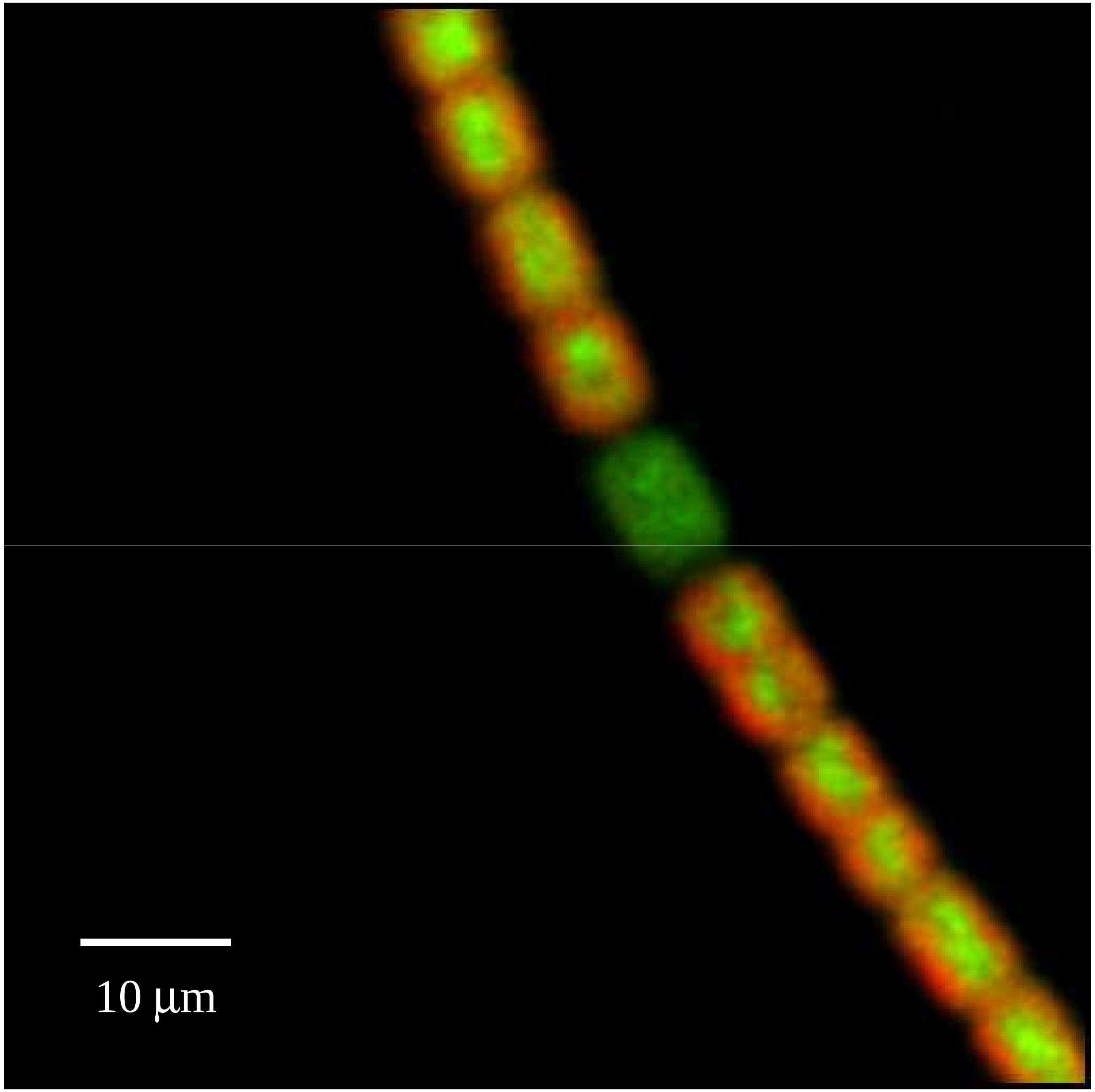
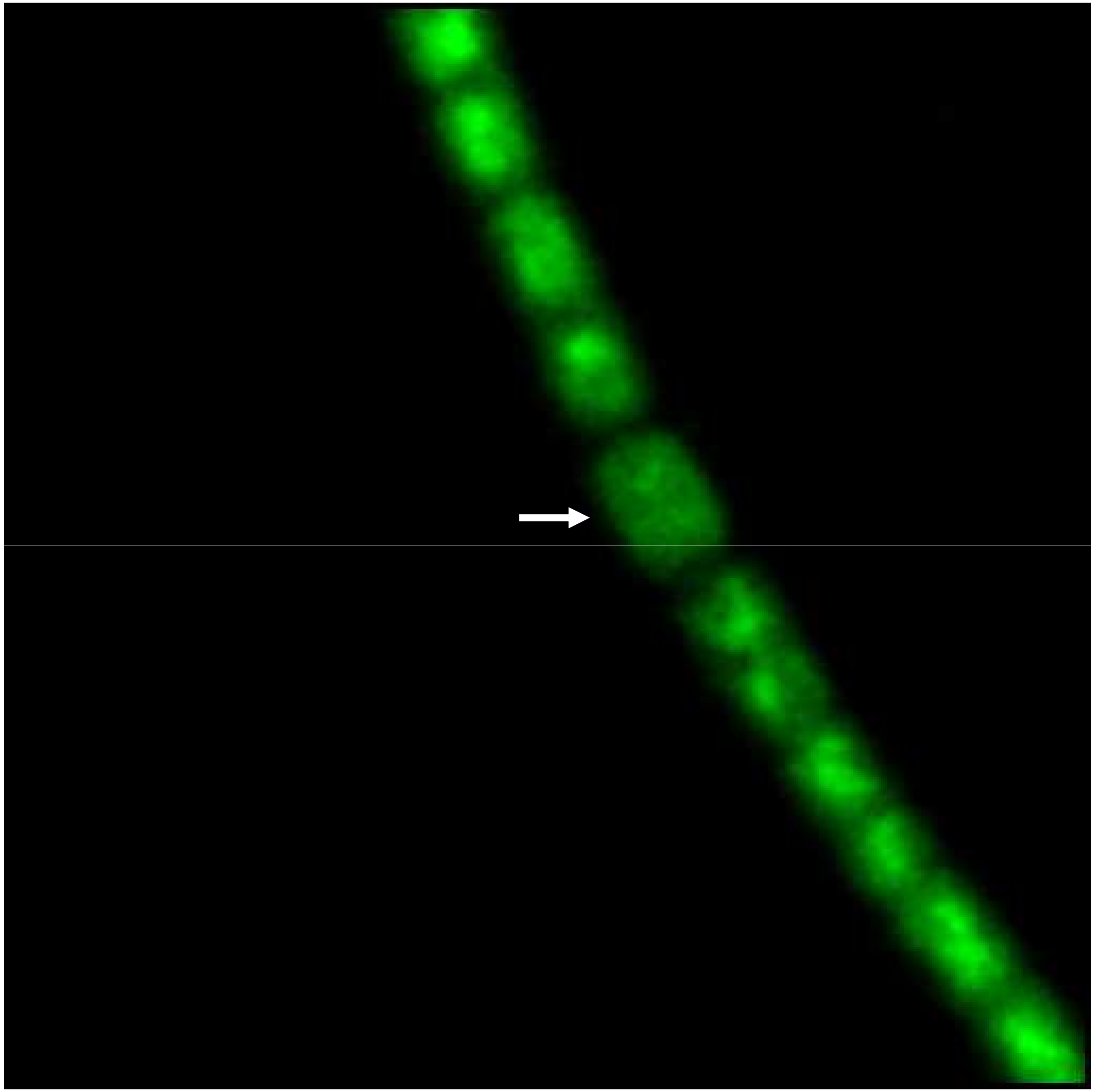
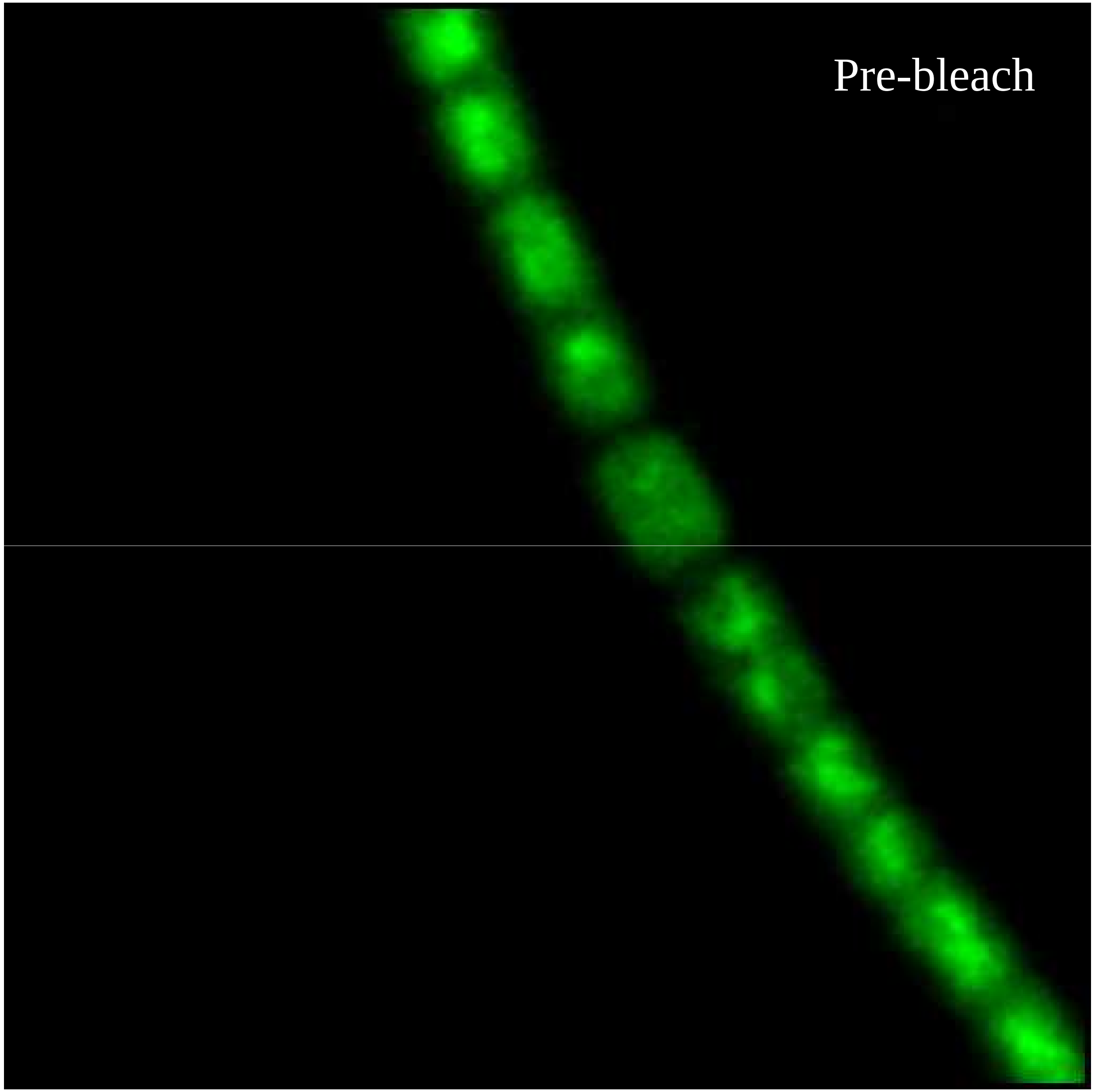
Calcein FRAP in *Anabaena cylindrica* (N_2-fixing)



Dye exchange between vegetative cells and heterocyst

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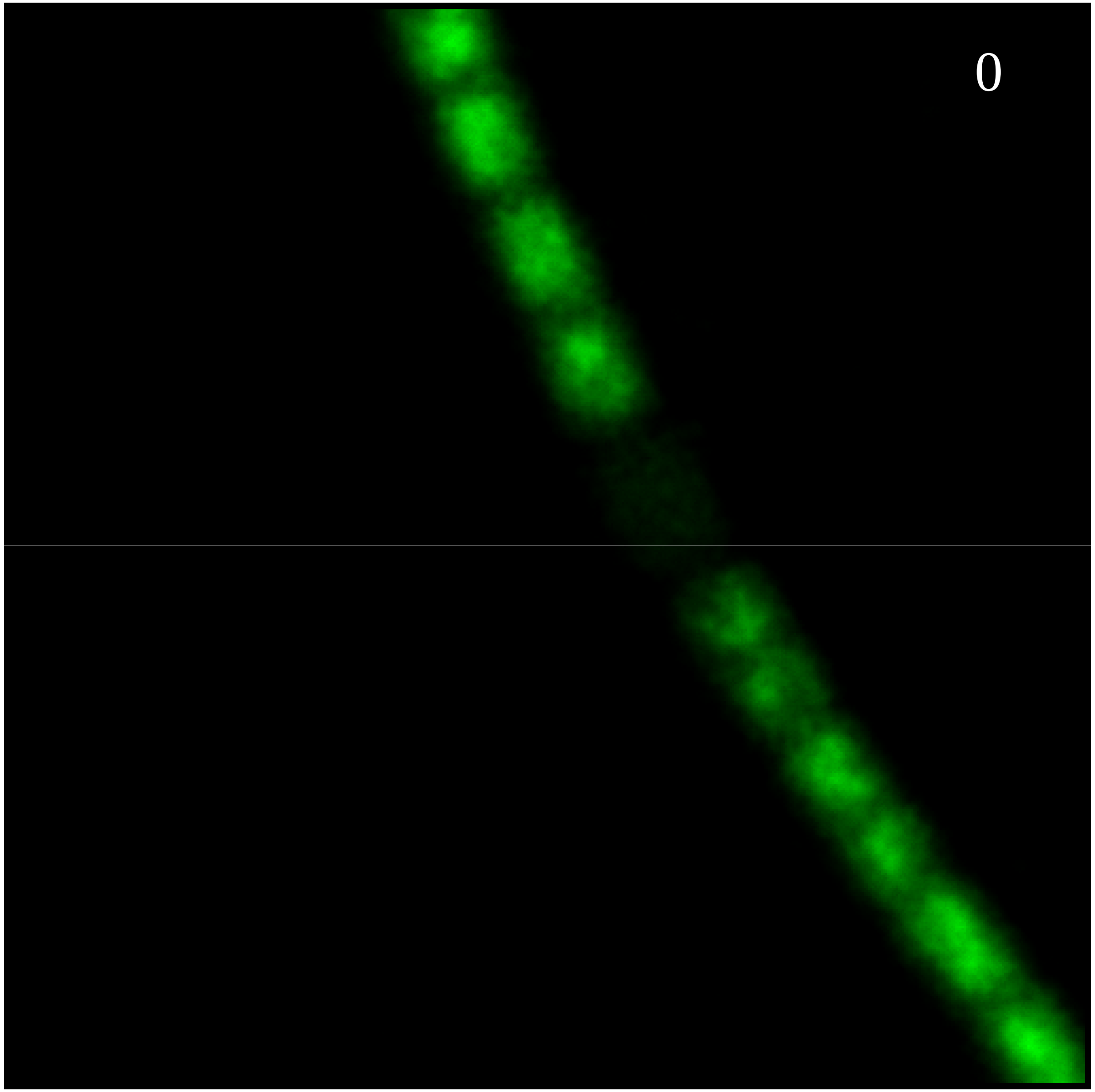
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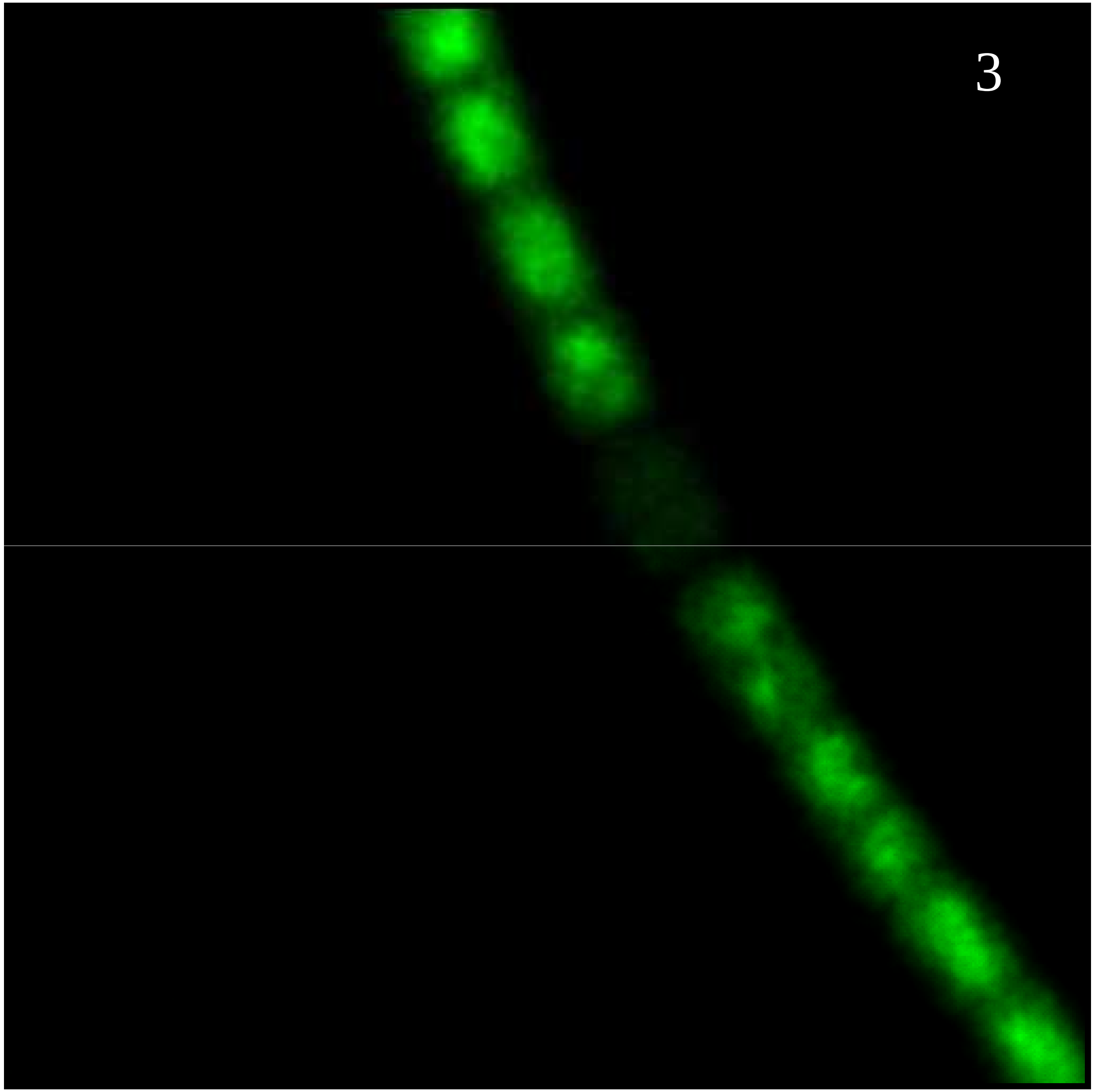
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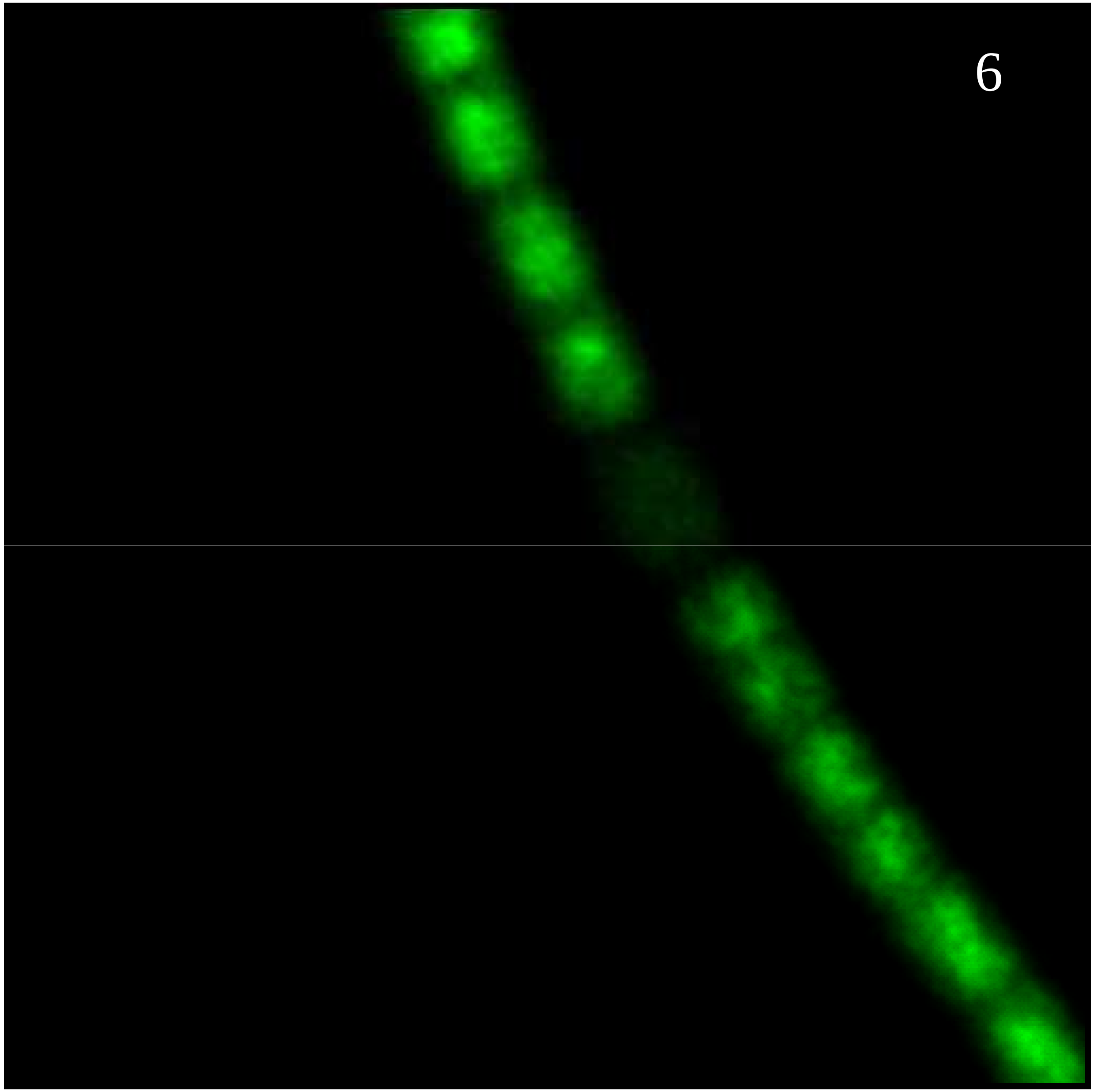
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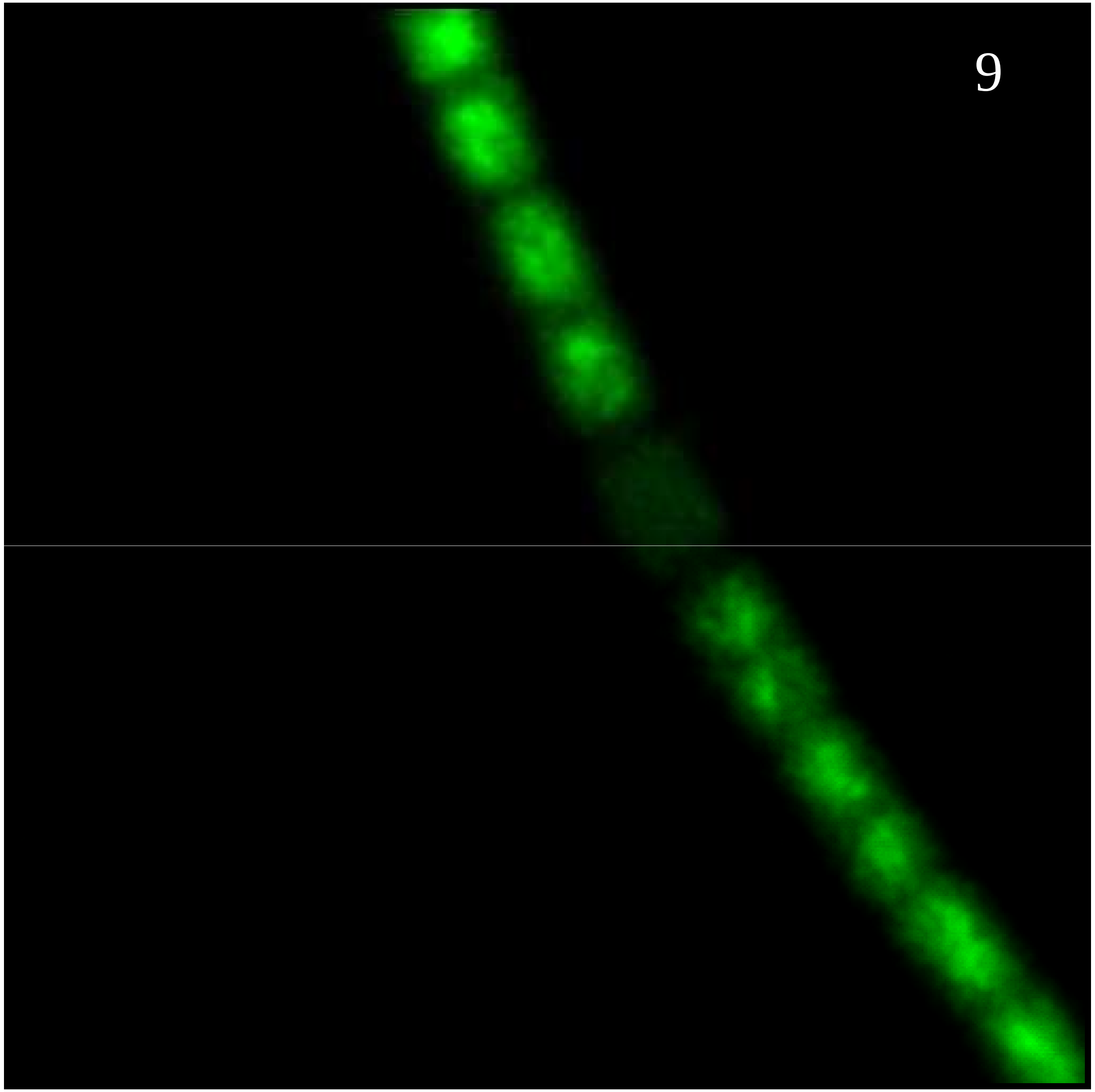
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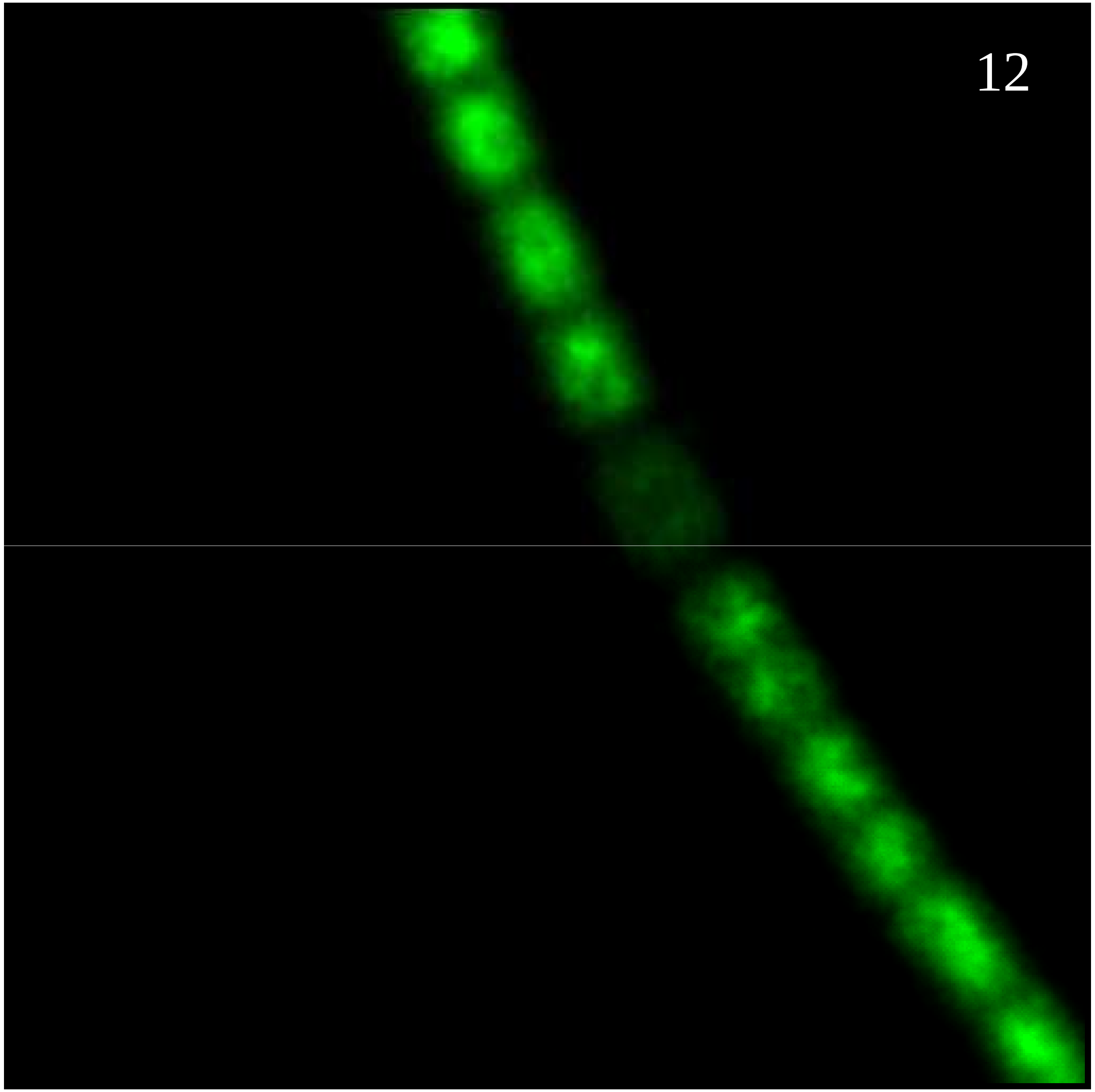
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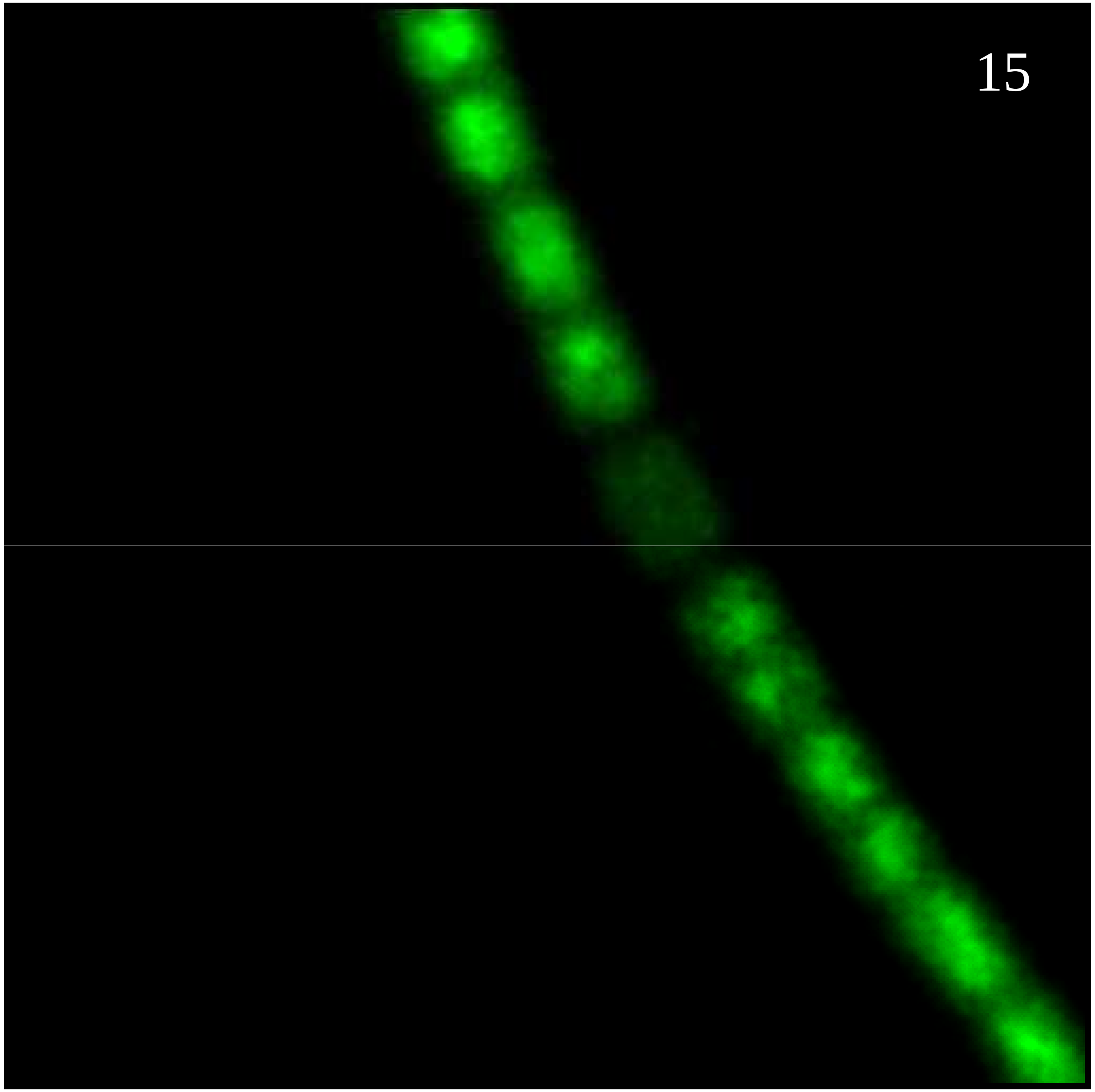
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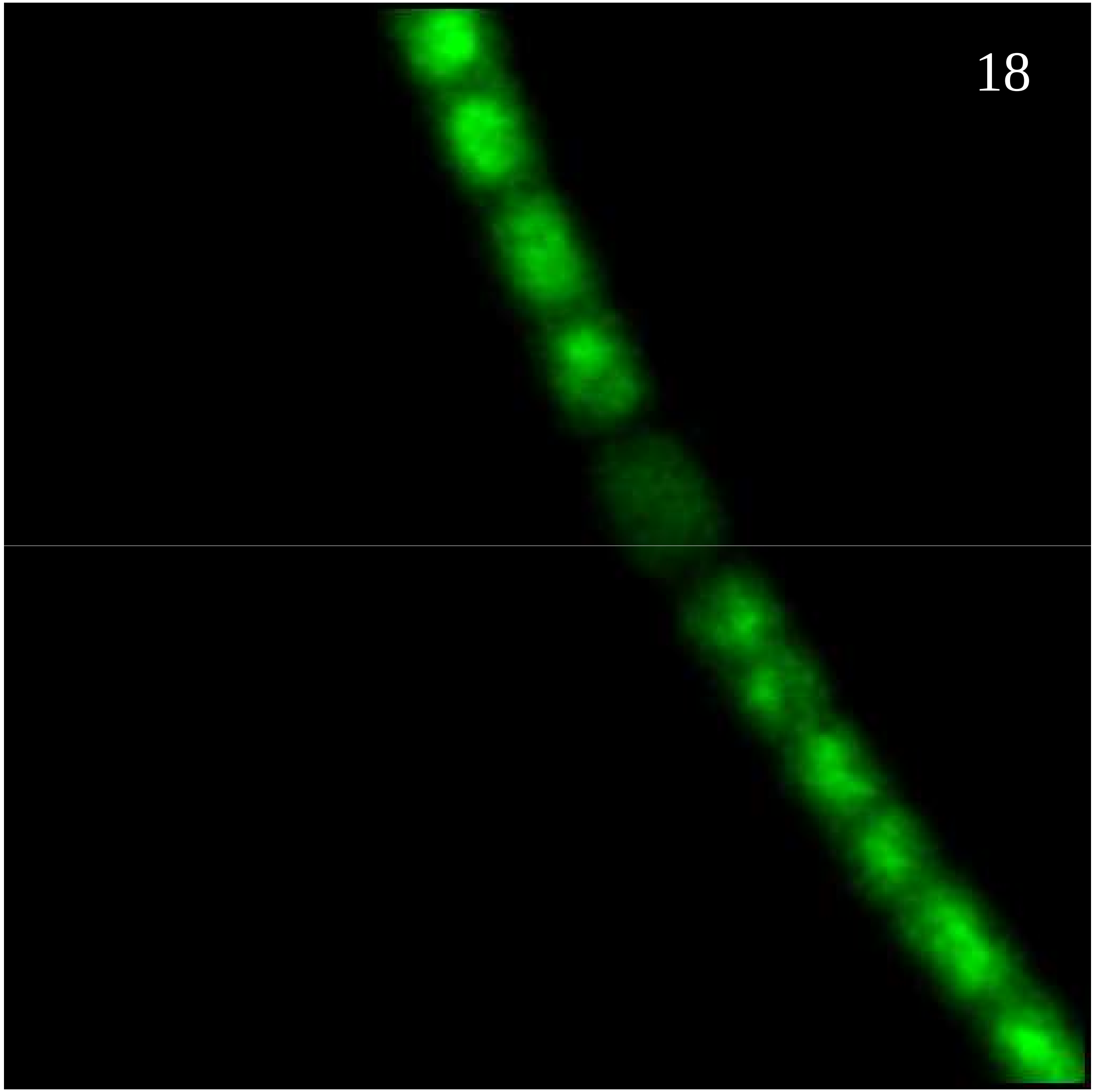
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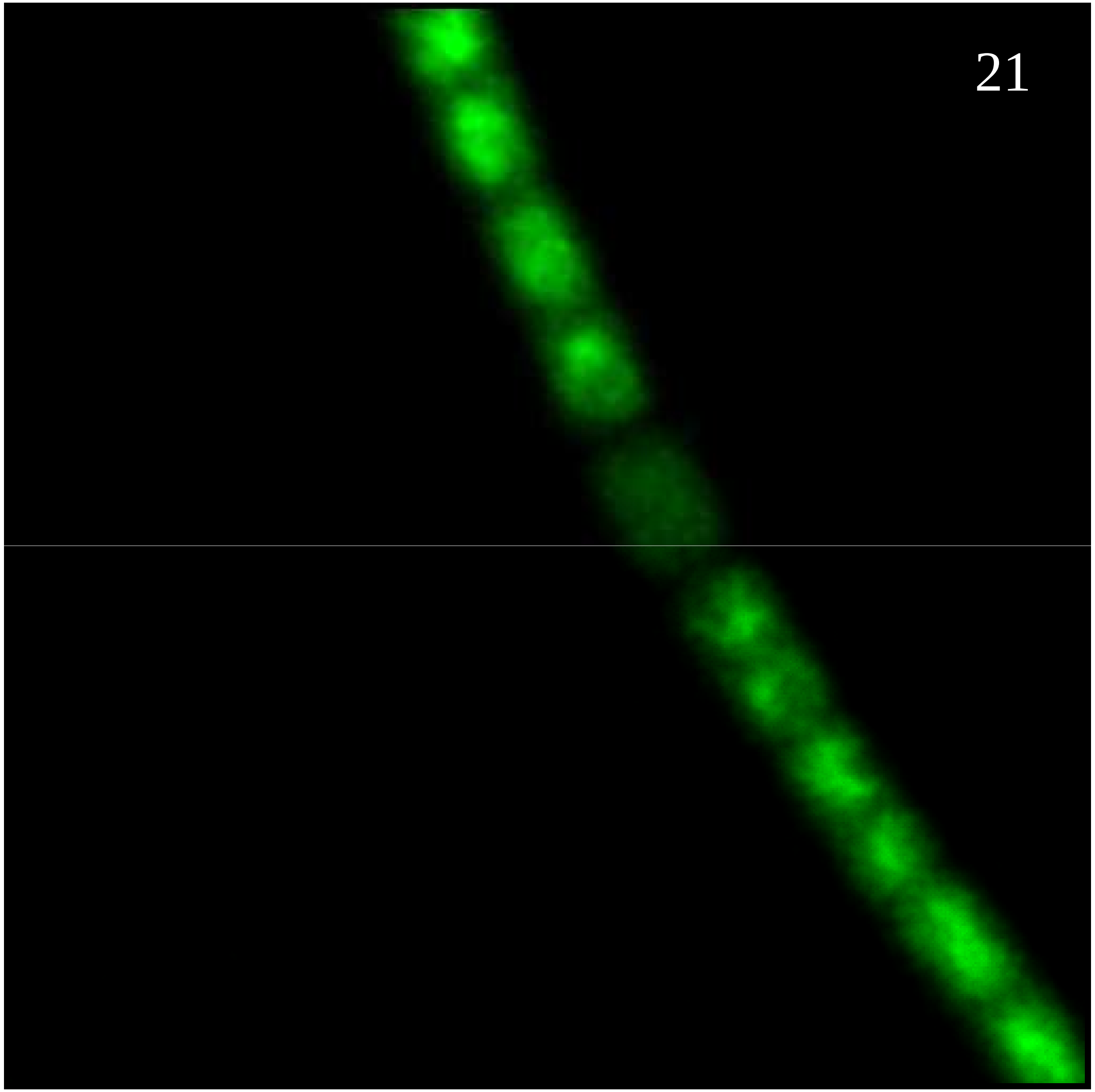
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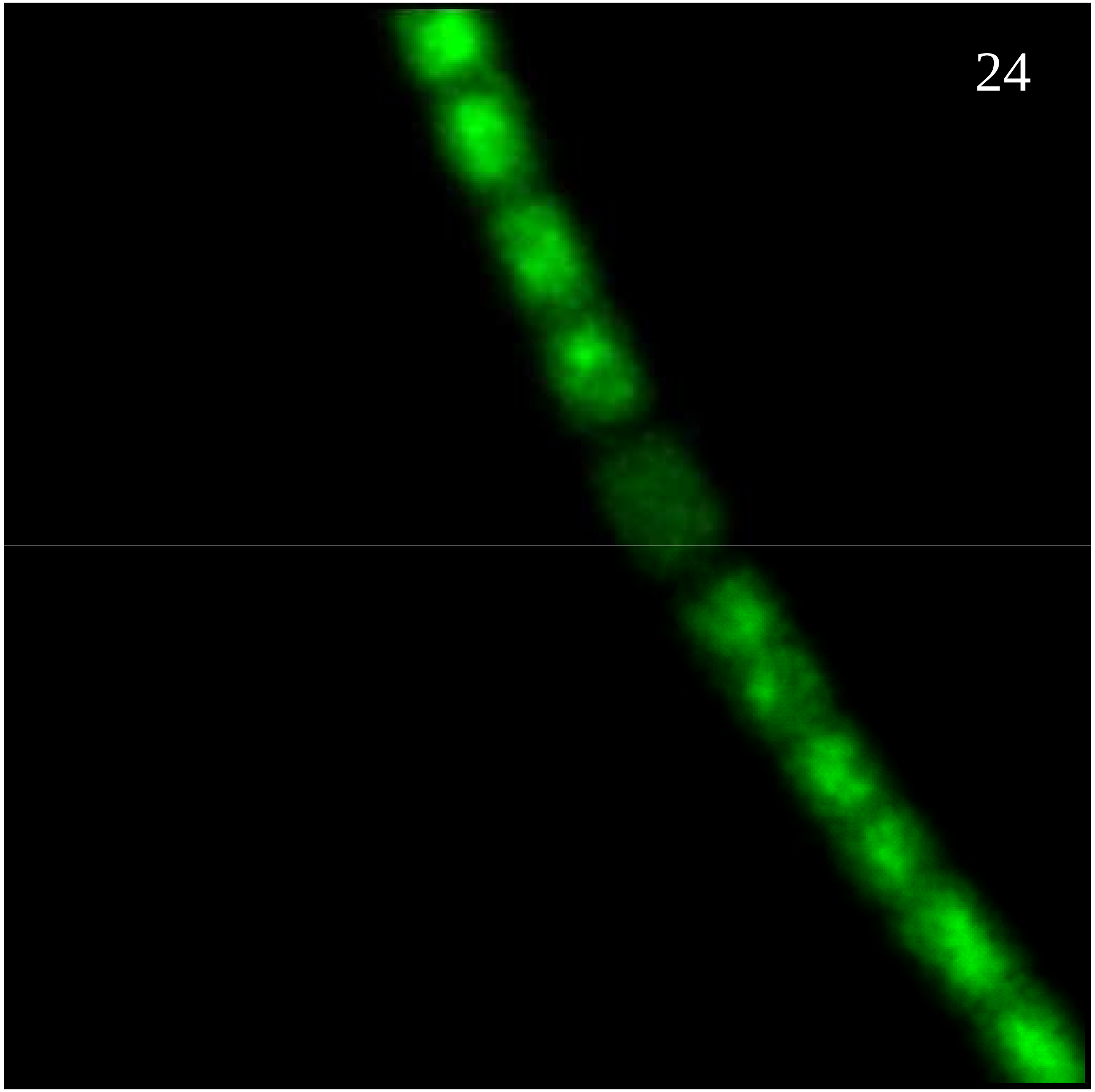
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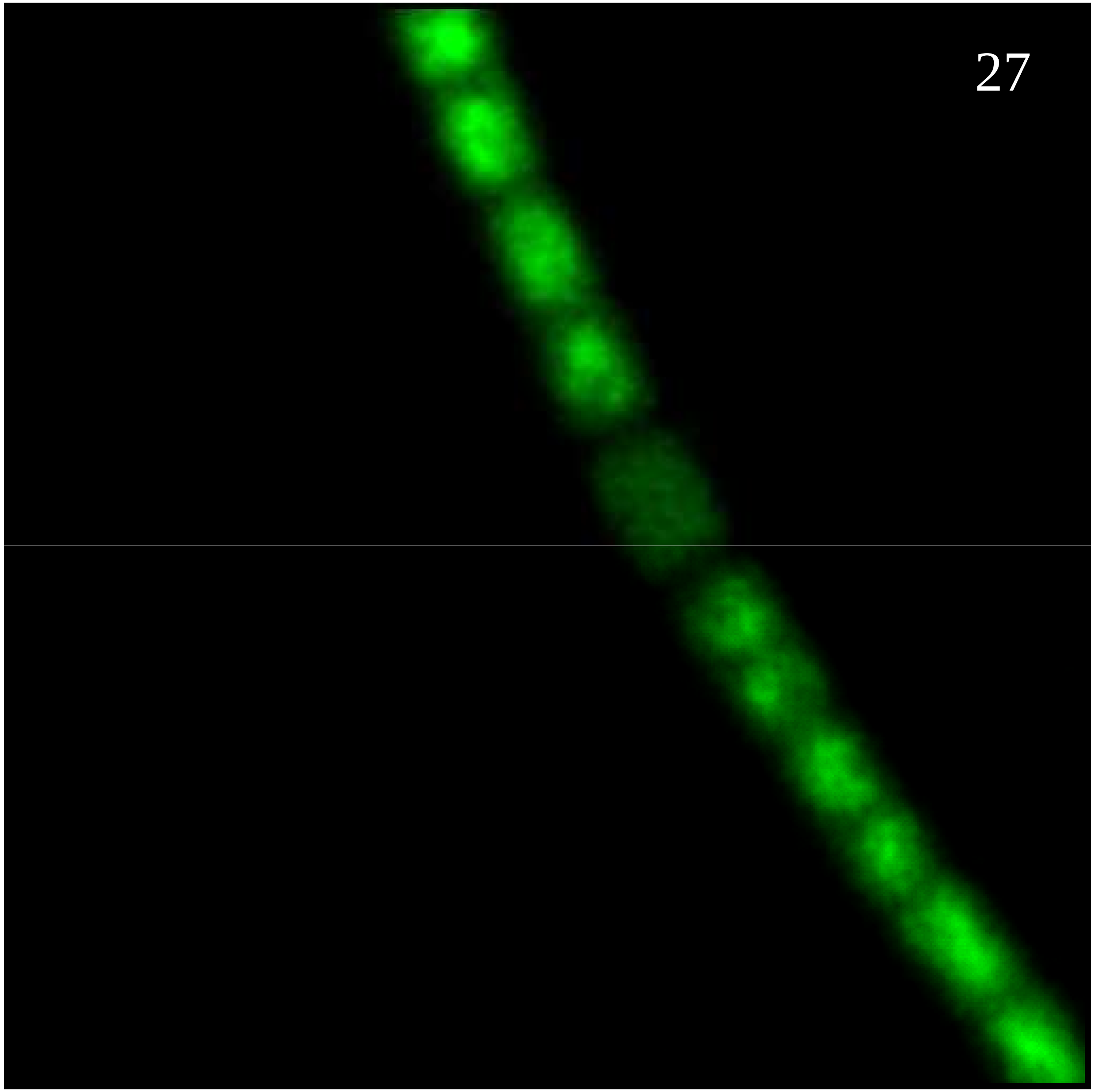
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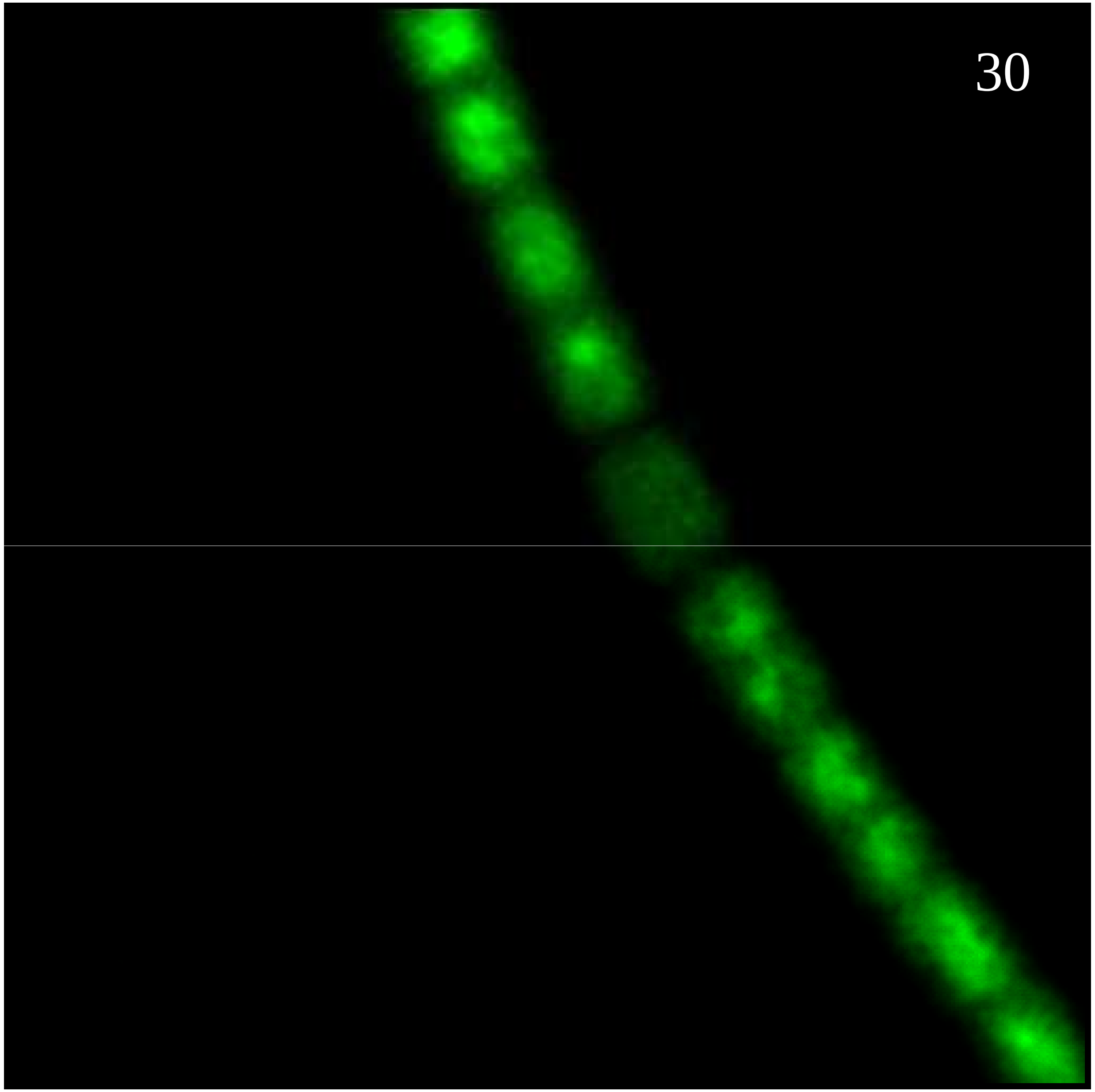
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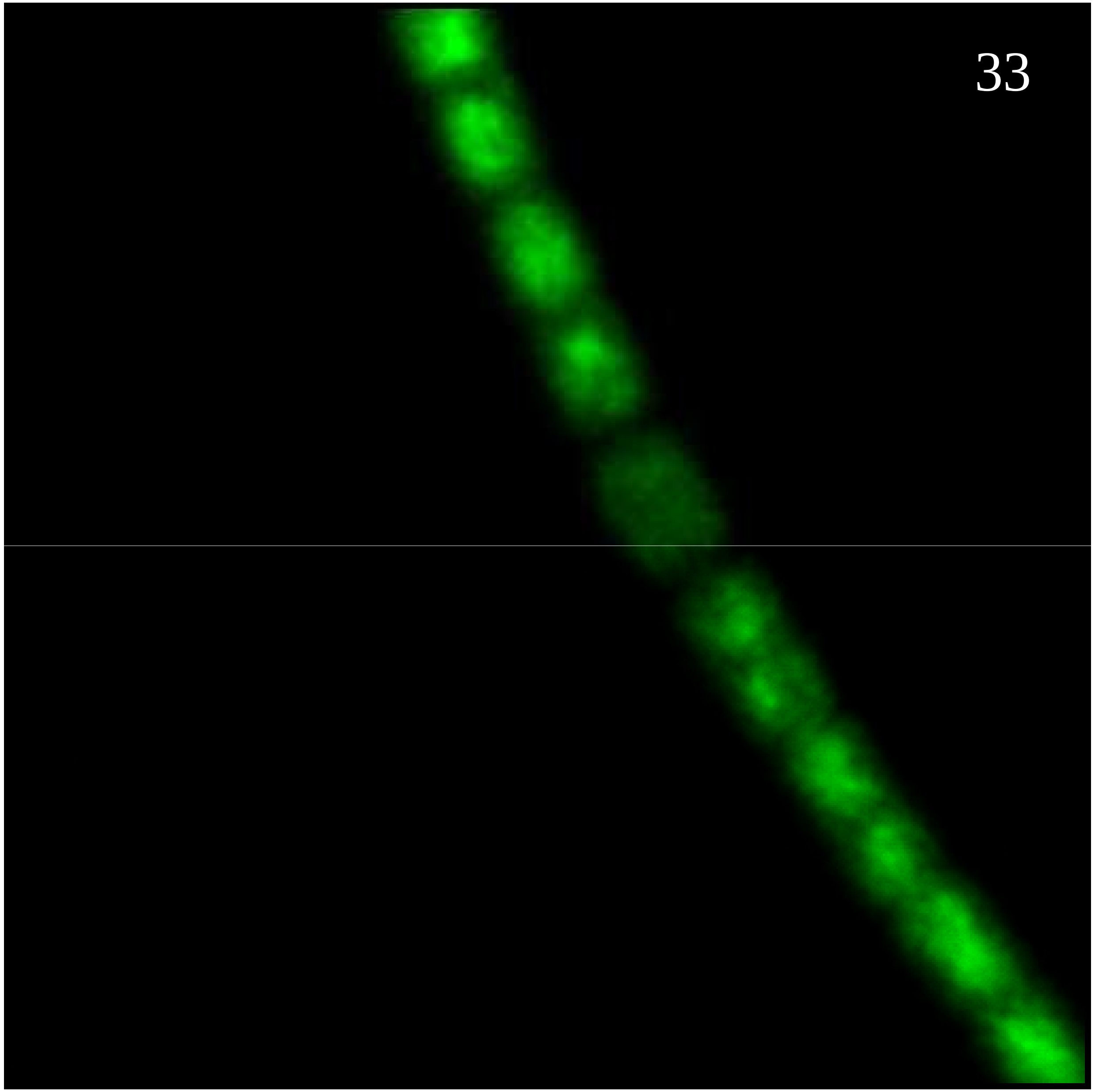
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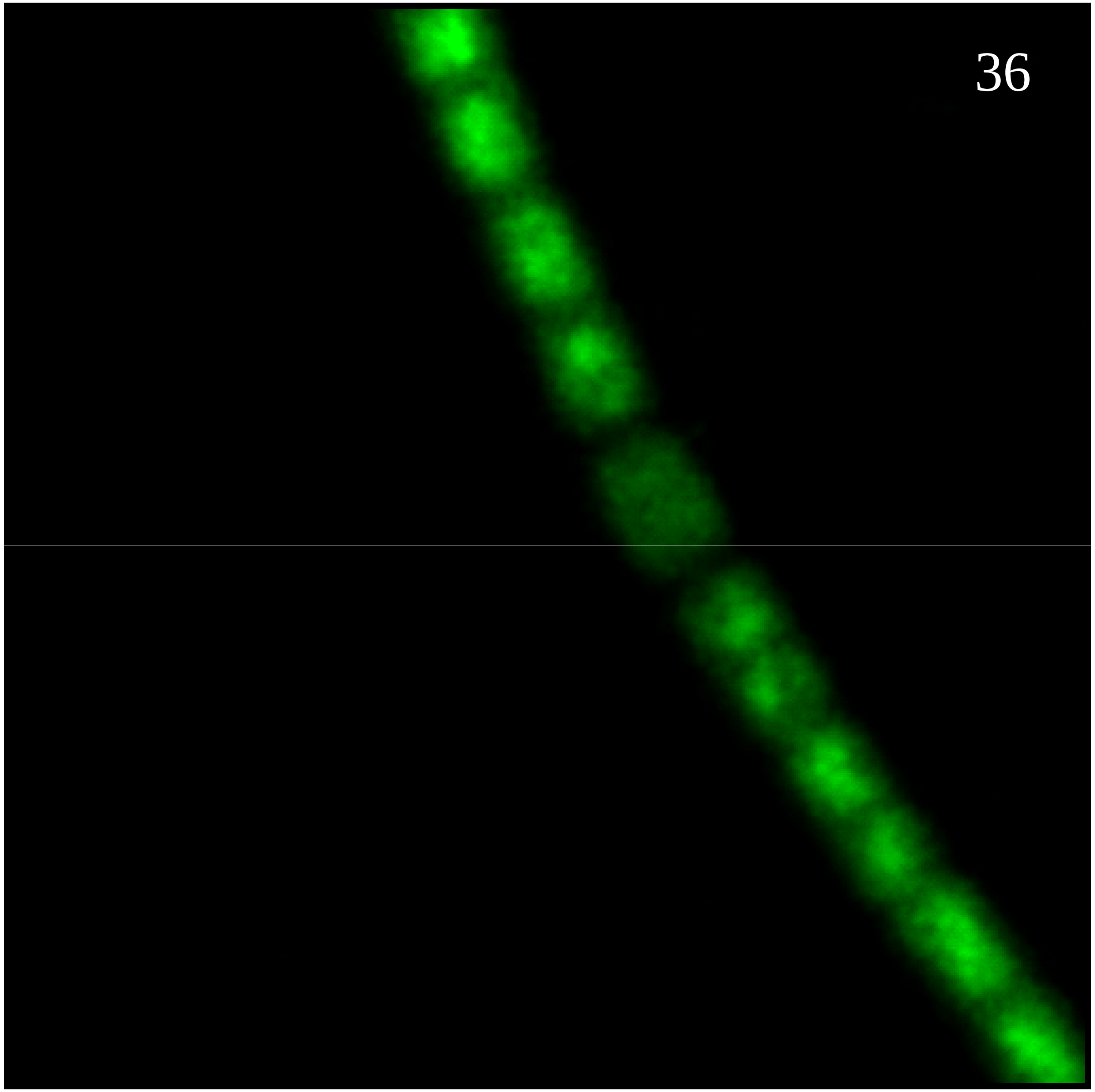
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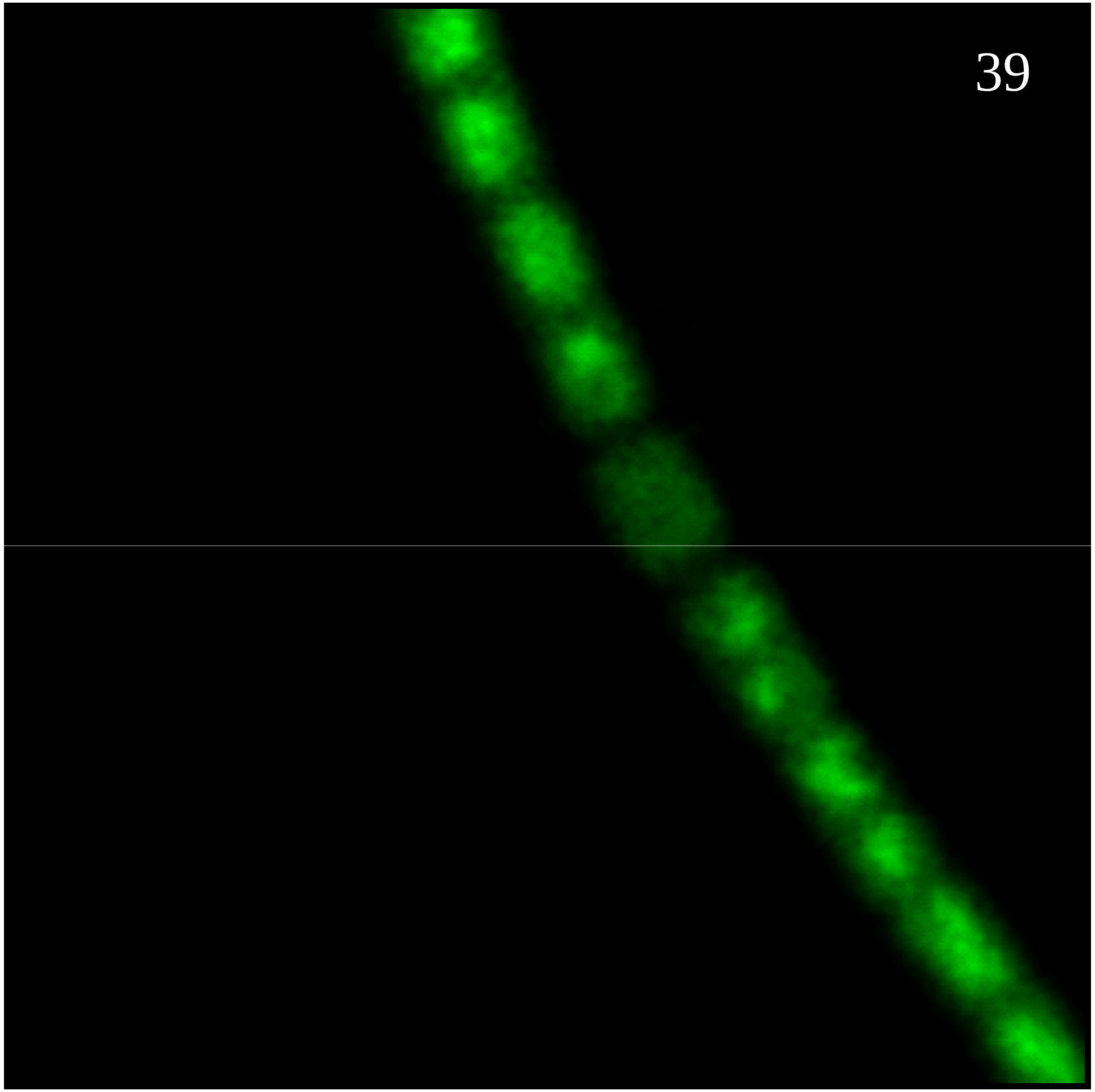
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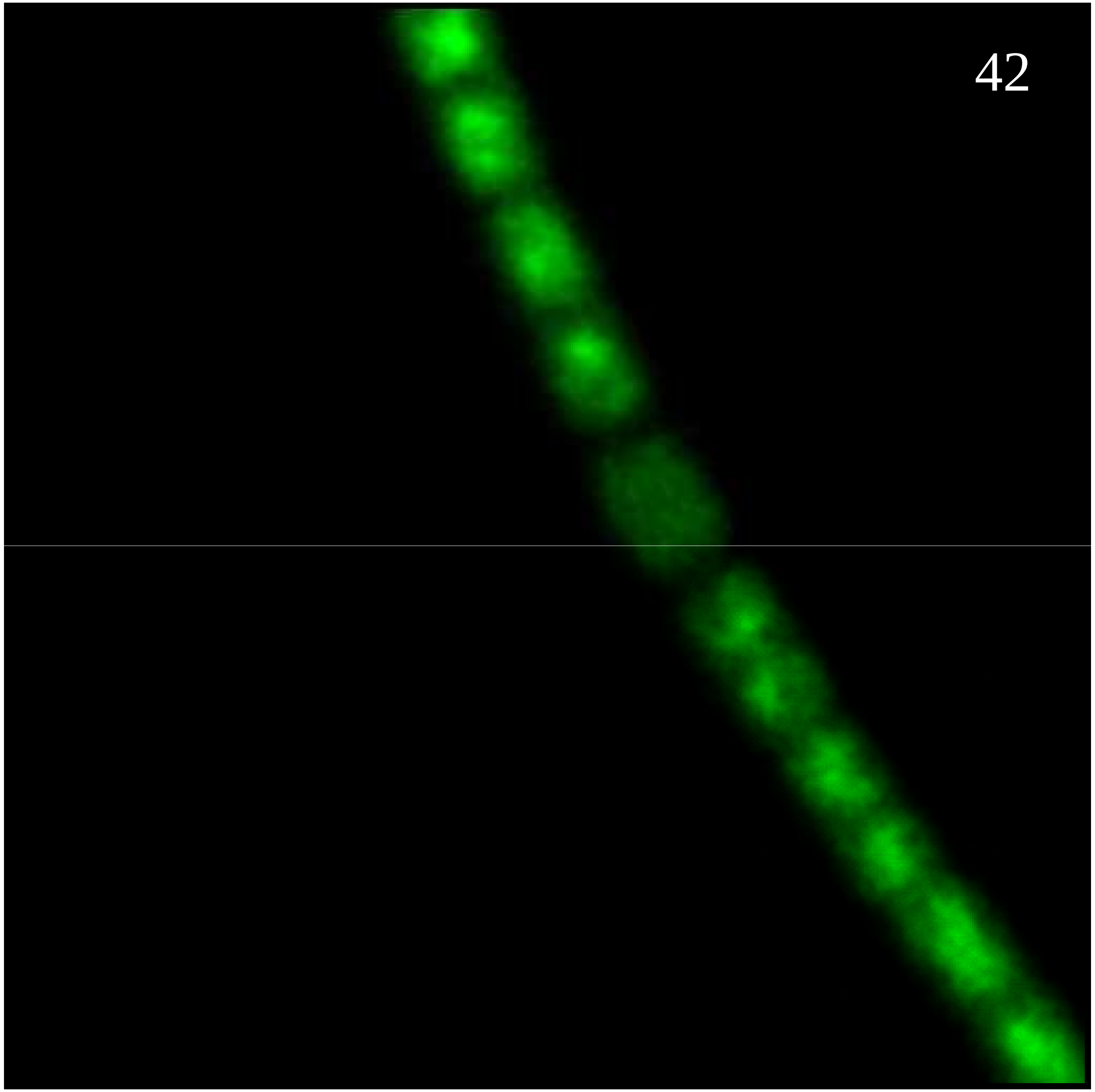
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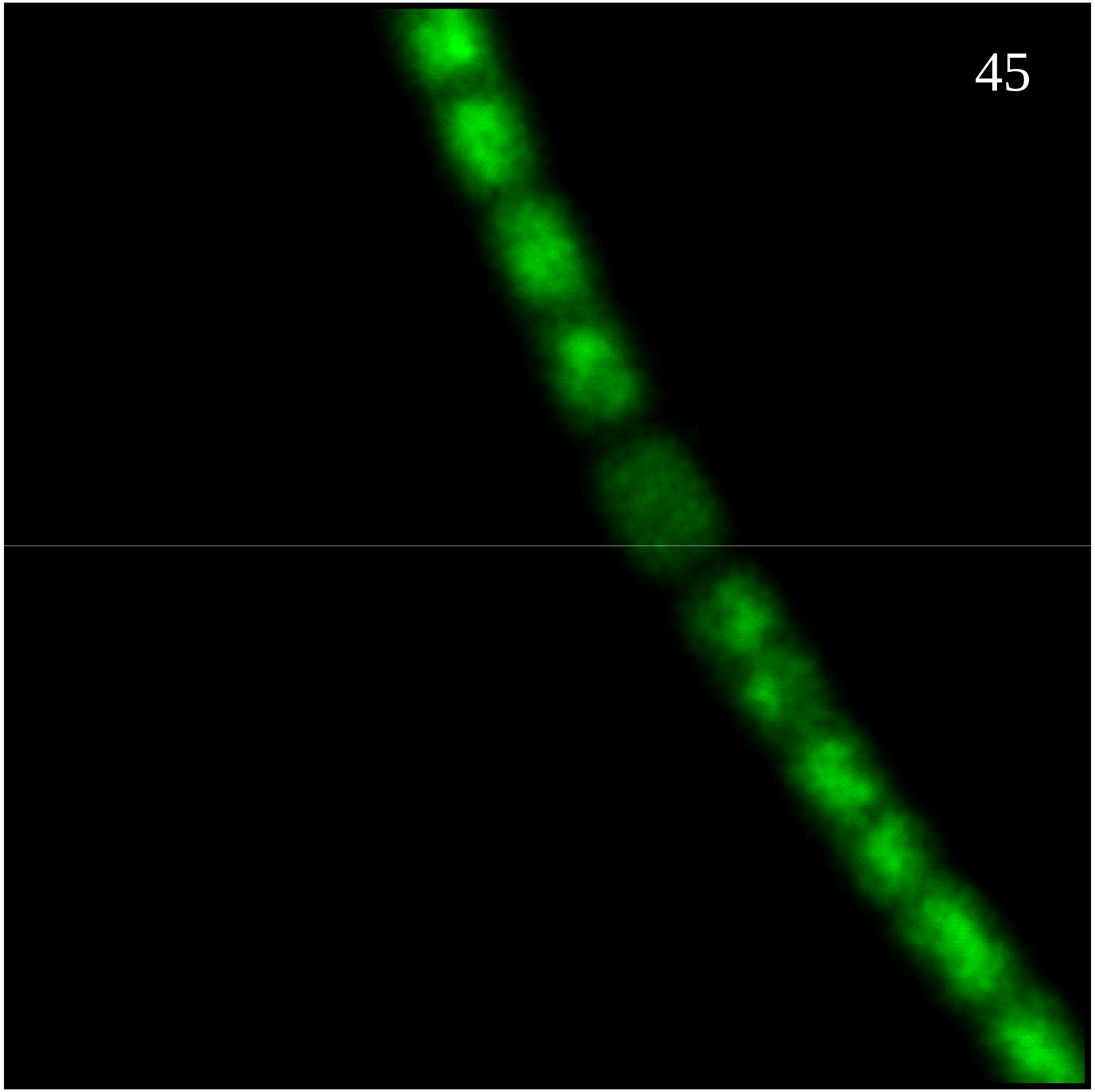
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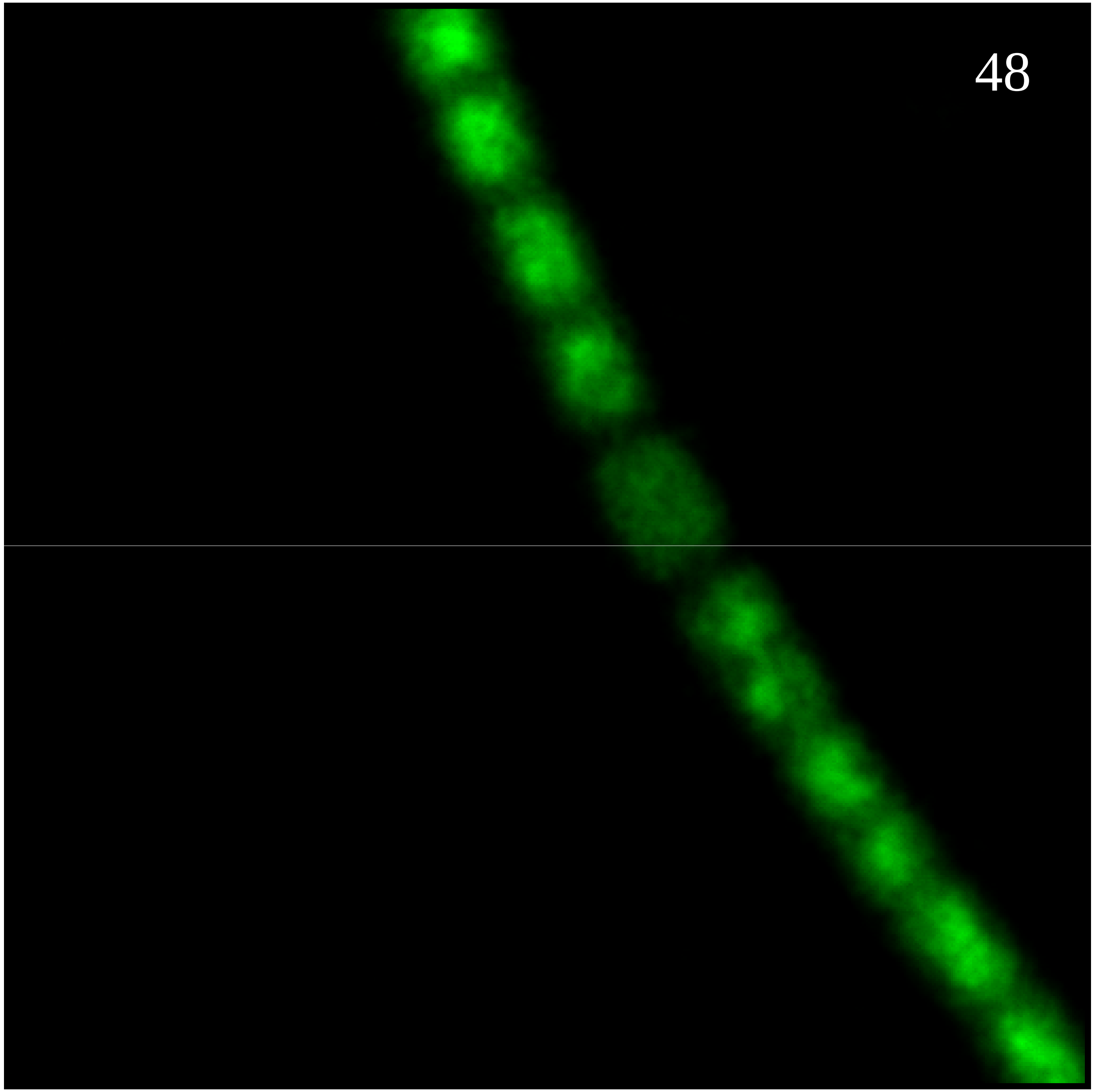
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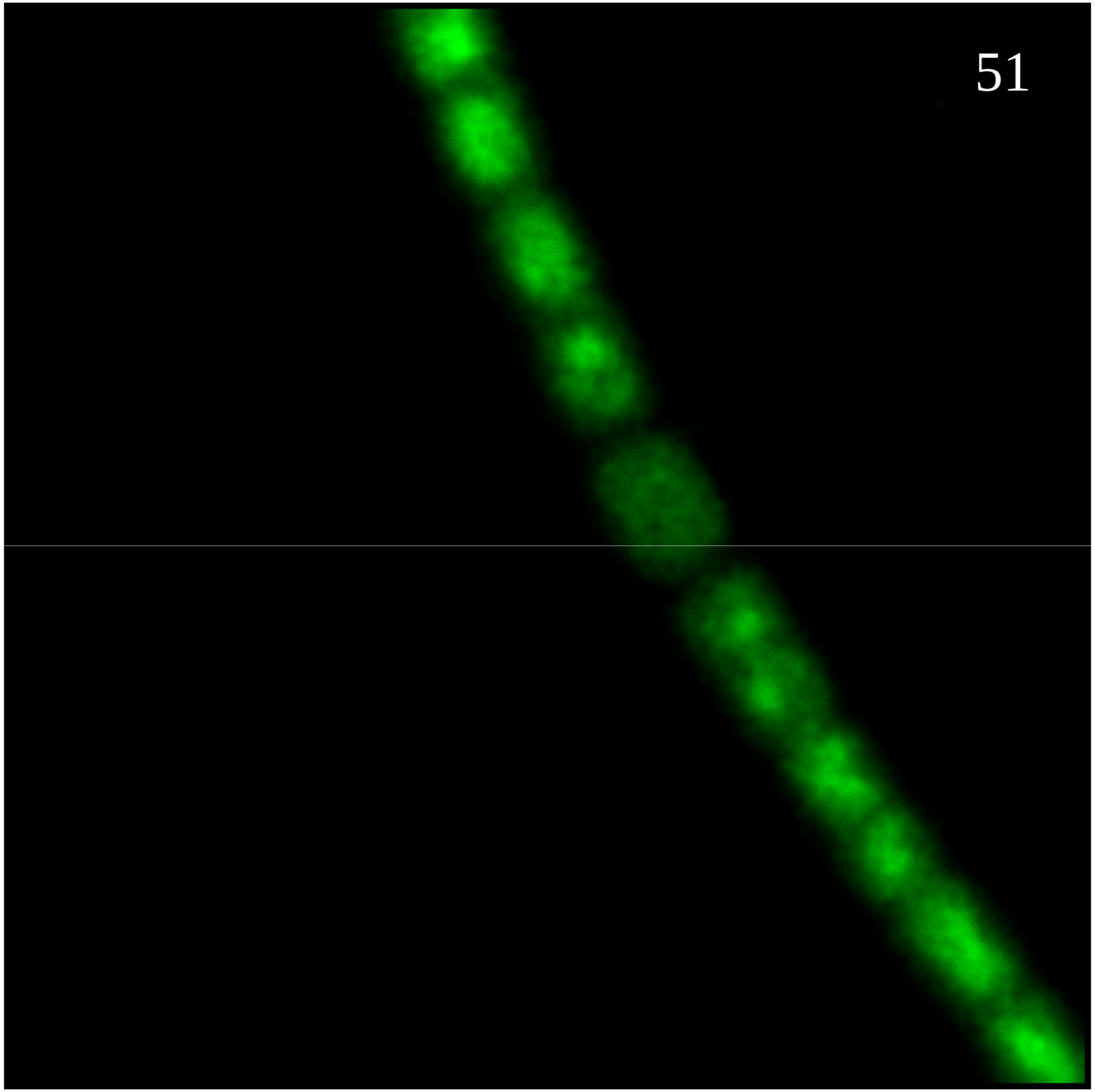
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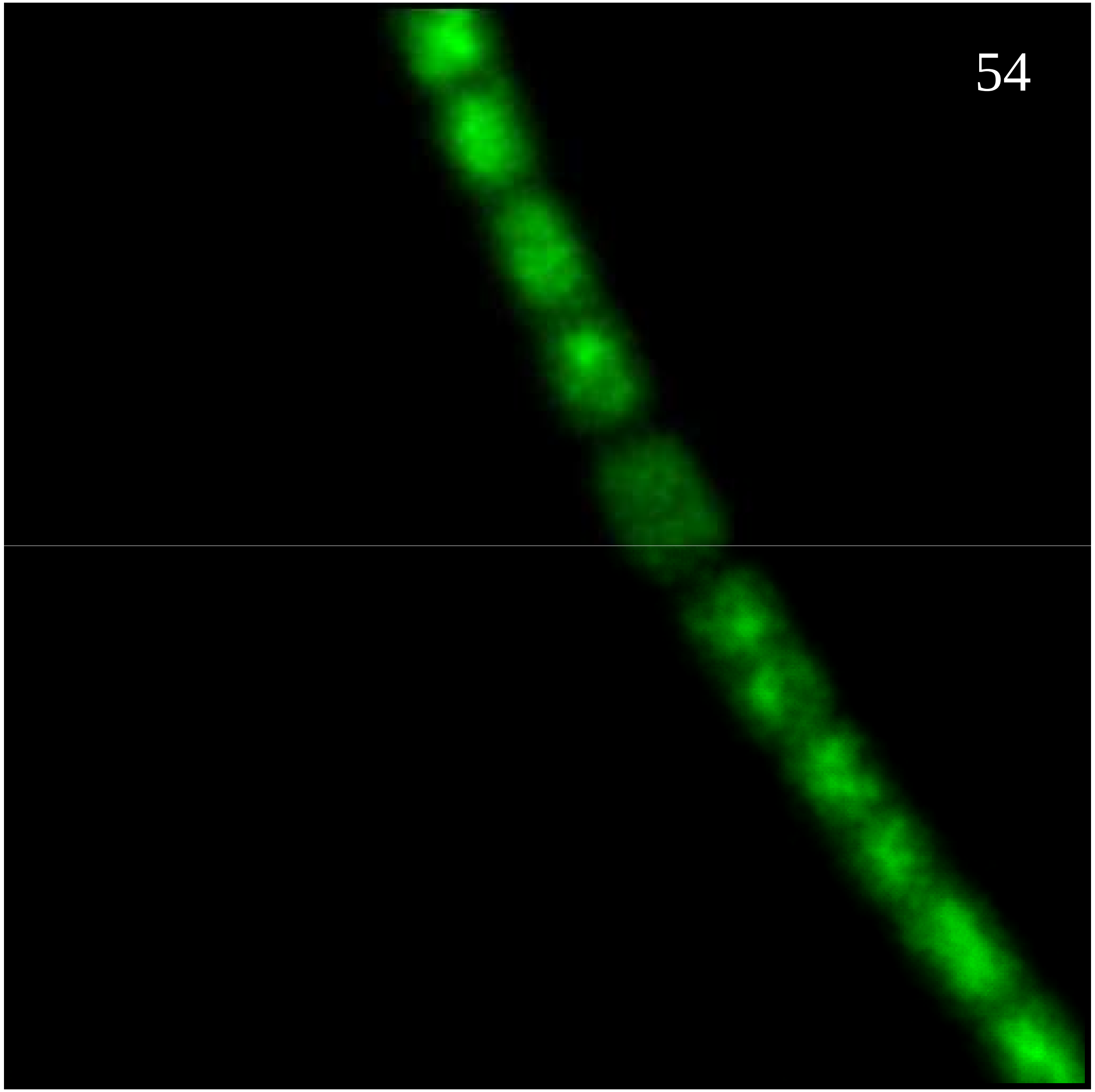
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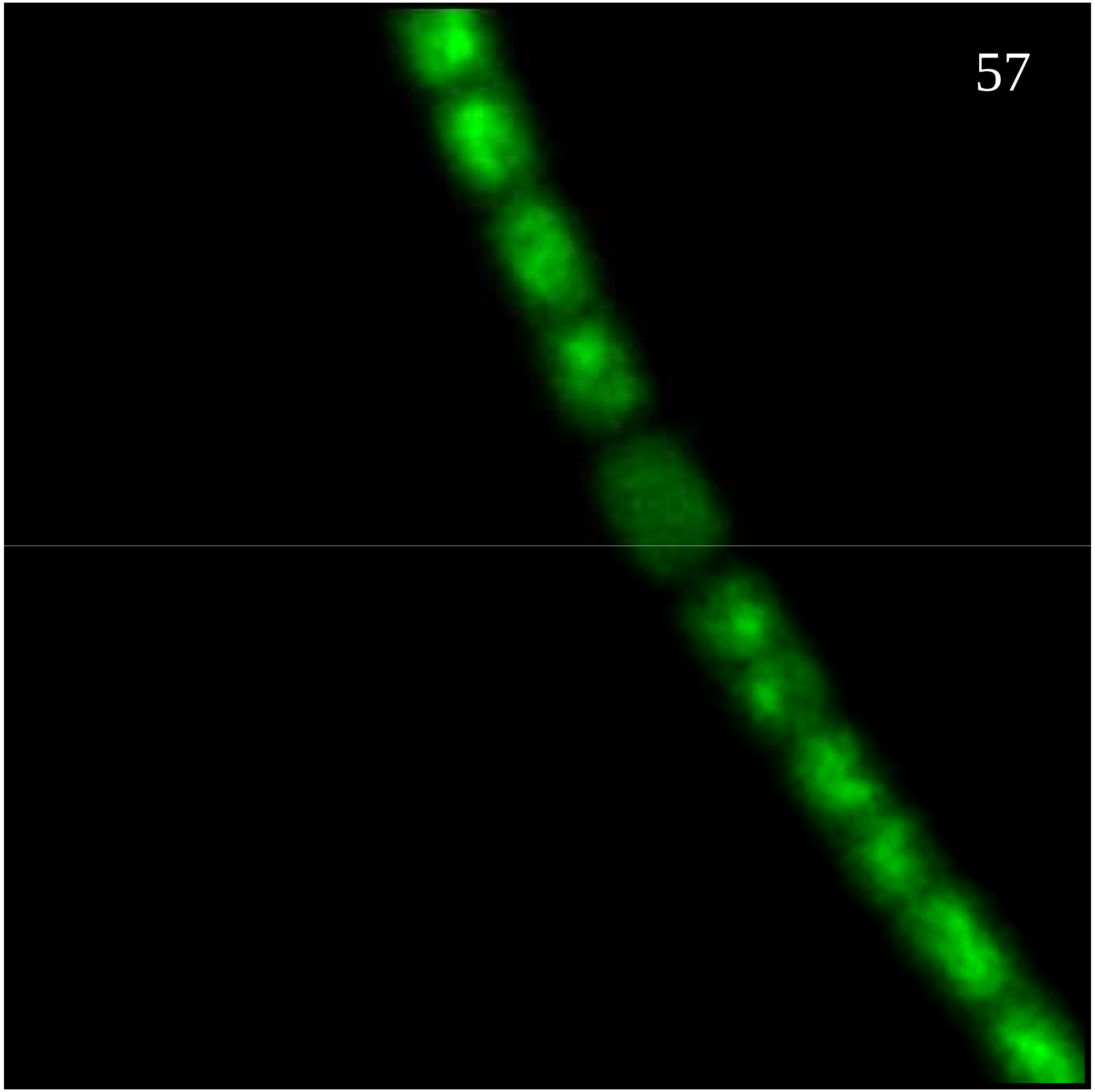
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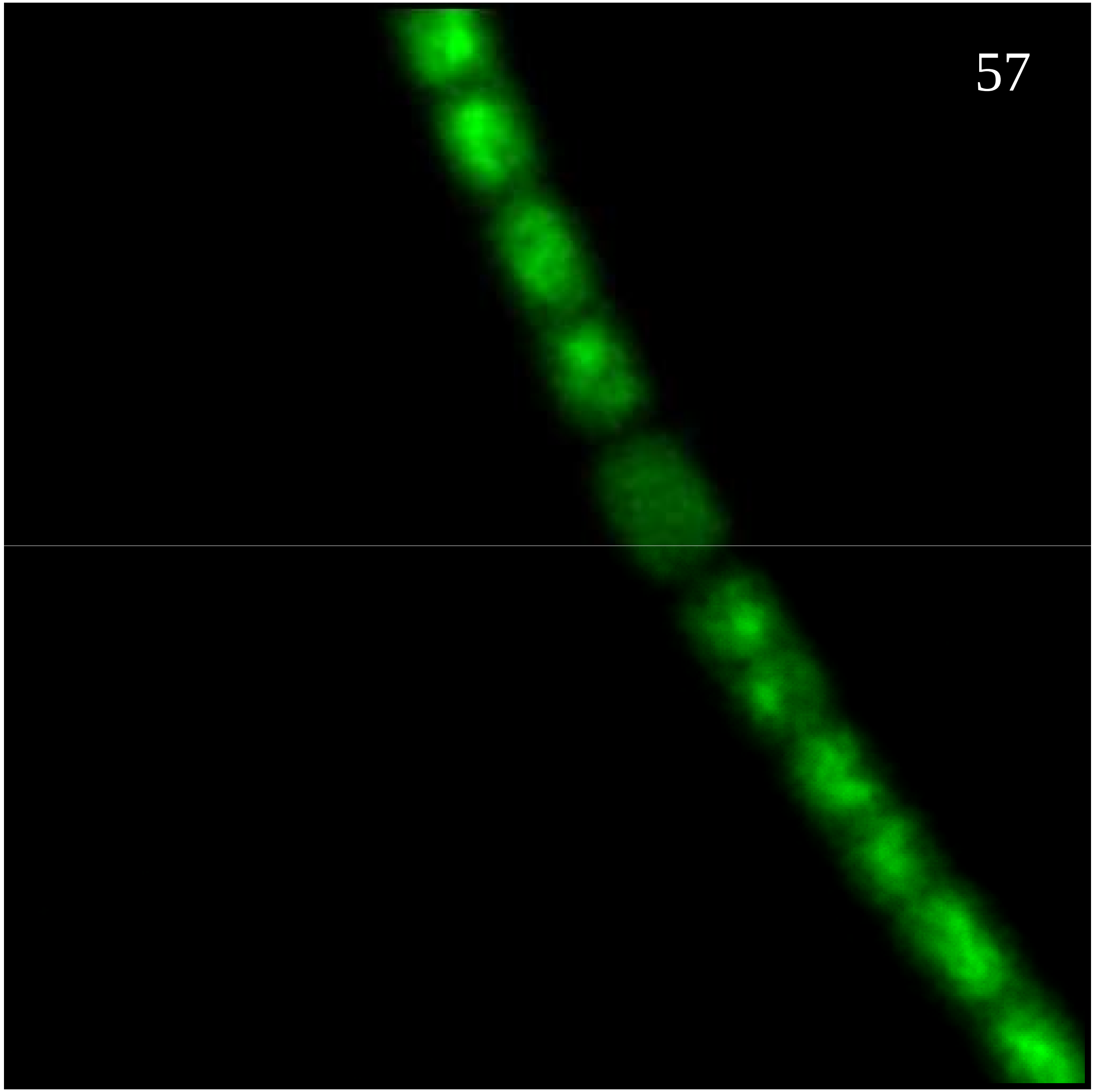
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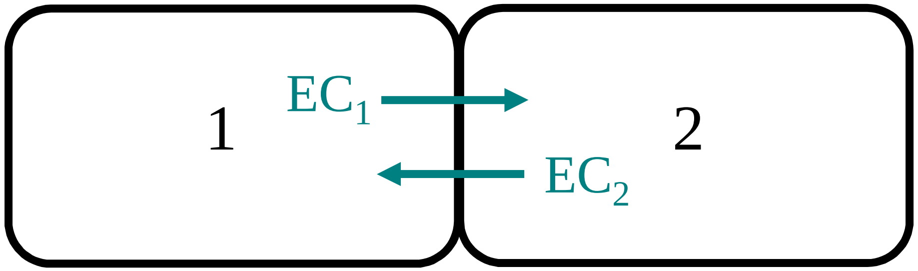
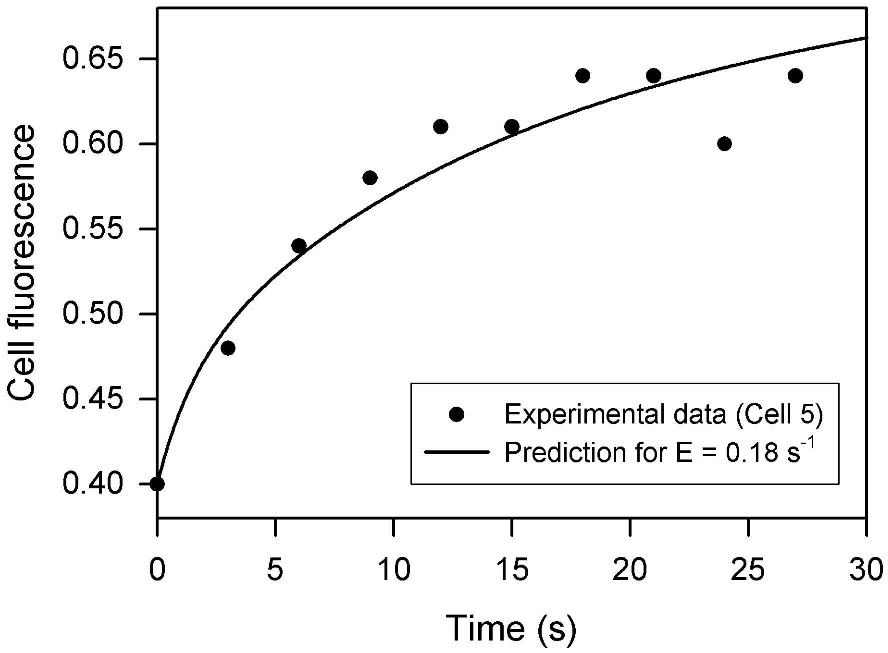
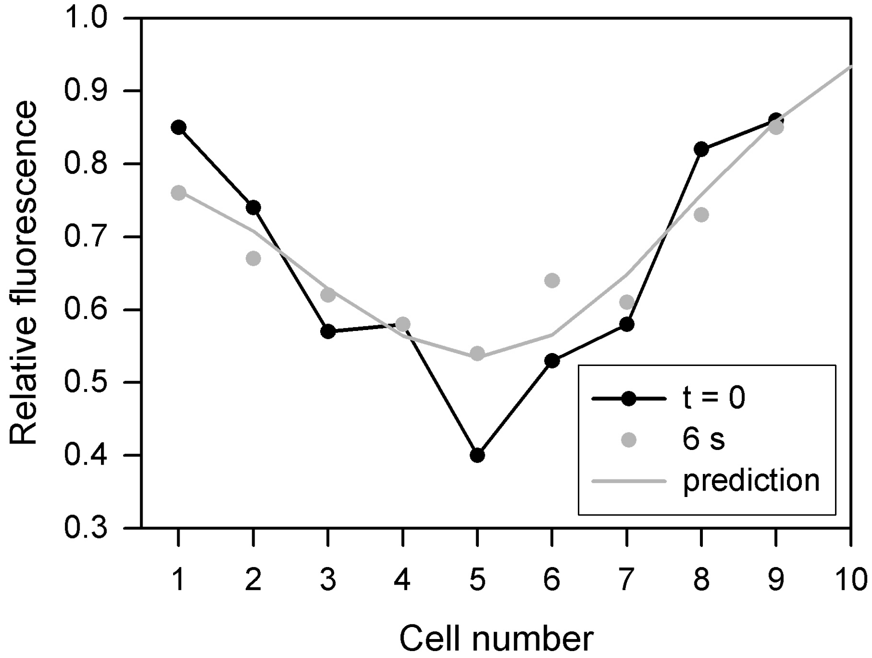
Data analysis in terms of an “exchange coefficient” E



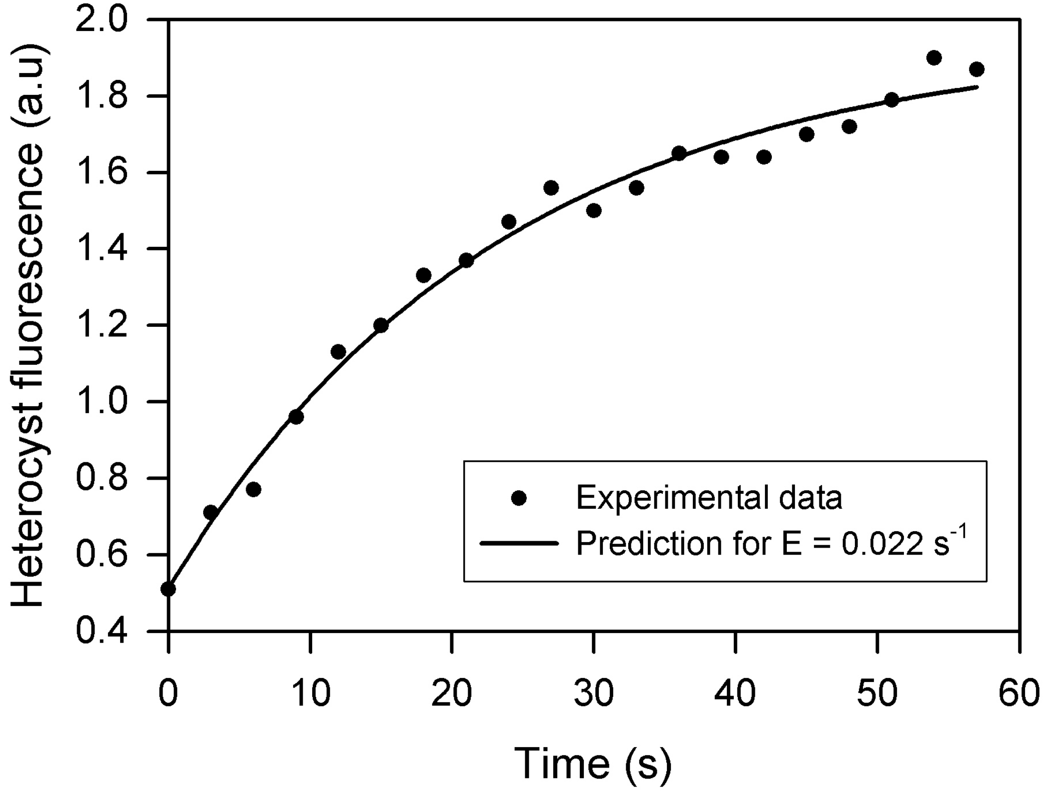
Net rate of exchange from Cell 1 to Cell 2 = $E (C_1 - C_2)$

Estimating E (veg-veg exchange)





Estimating E (veg-het exchange)



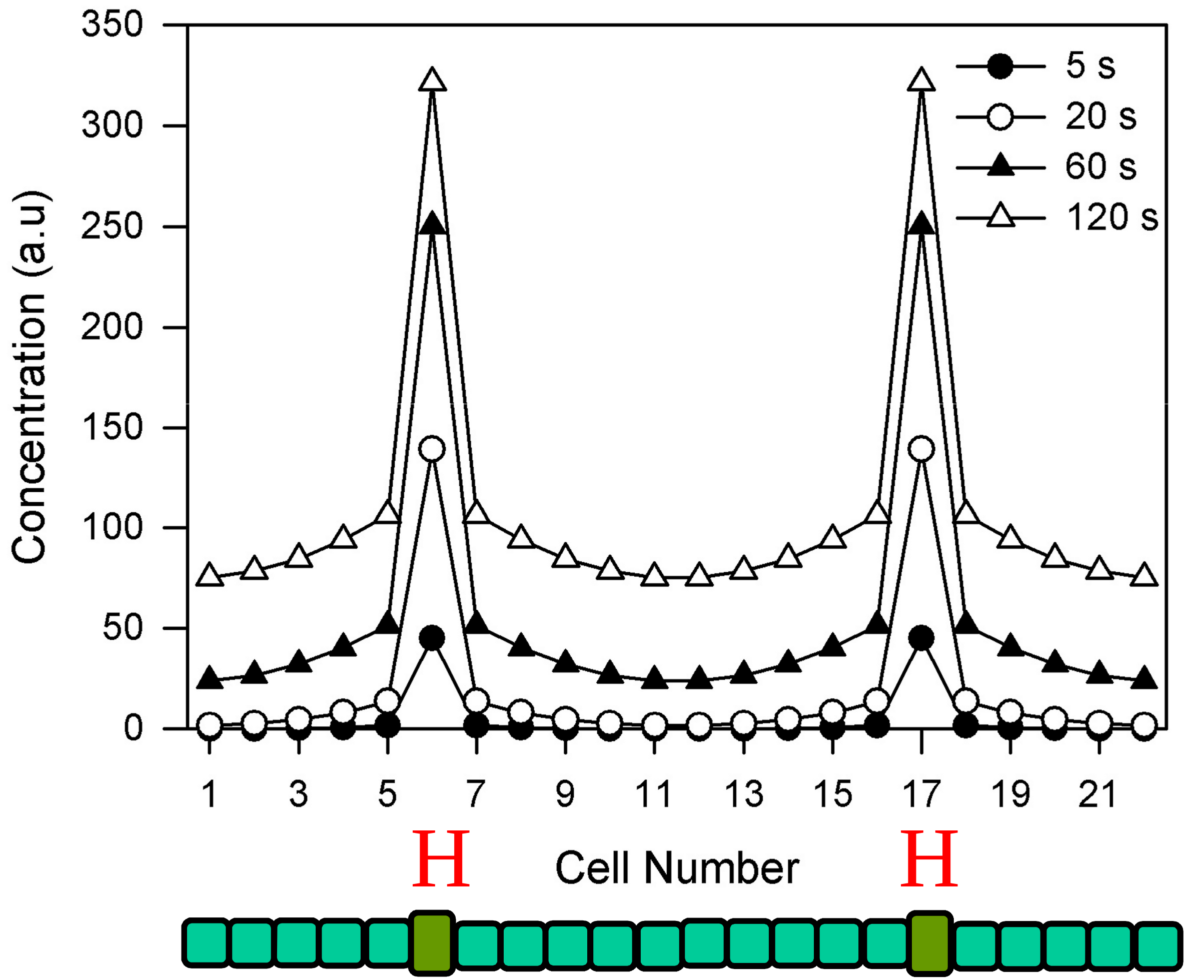
Mean E values for *Anabaena cylindrica* (s^{-1})

Nitrate-grown	0.11 ± 0.03	
18 hours - nitrate	0.14 ± 0.05	0.034 ± 0.011
72 hours - nitrate	0.29 ± 0.13	0.022 ± 0.011
	Vegetative cells	(Pro) heterocysts

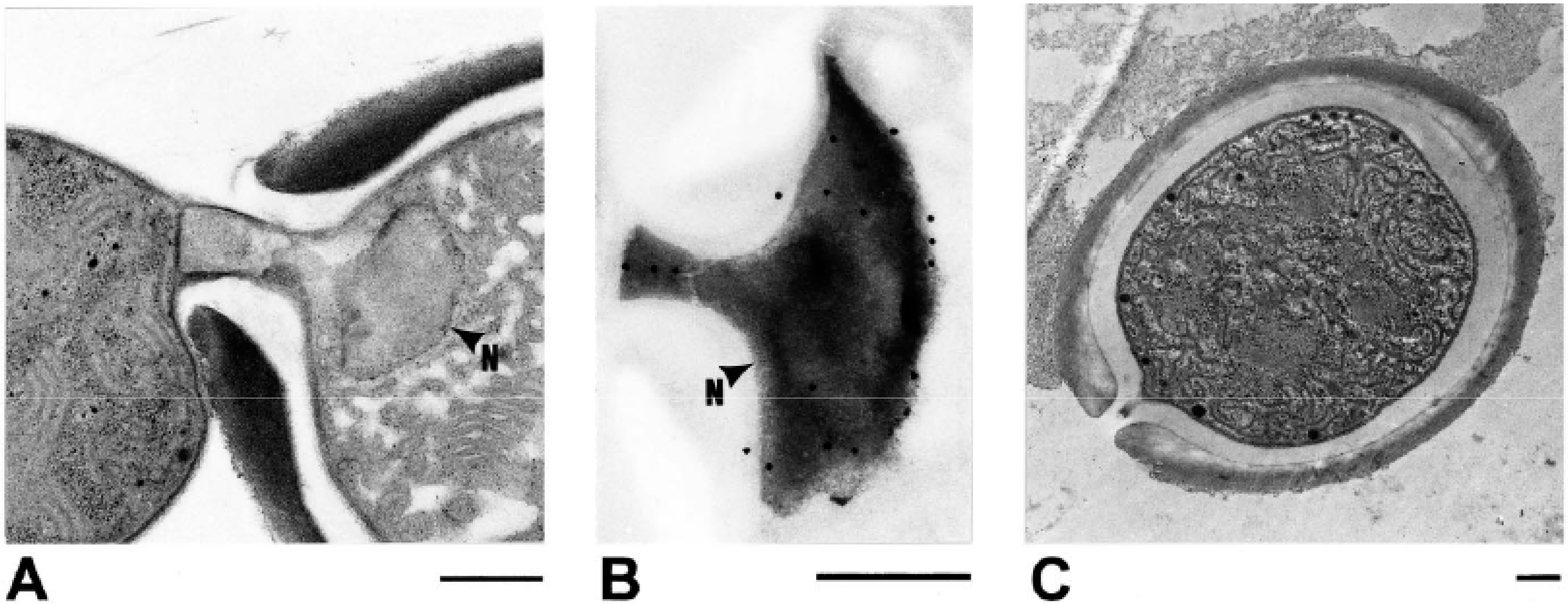
Mean E values for *Anabaena* PCC7120 (s^{-1})

Nitrate-grown	0.021 ± 0.011	
16 hours - nitrate	0.057 ± 0.039	
96 hours - nitrate	0.28 ± 0.12	0.035 ± 0.012
	Vegetative cells	Heterocysts

Modelling the spread of a metabolite synthesised in the heterocysts (E values for fully-differentiated *Anabaena cylindrica*)



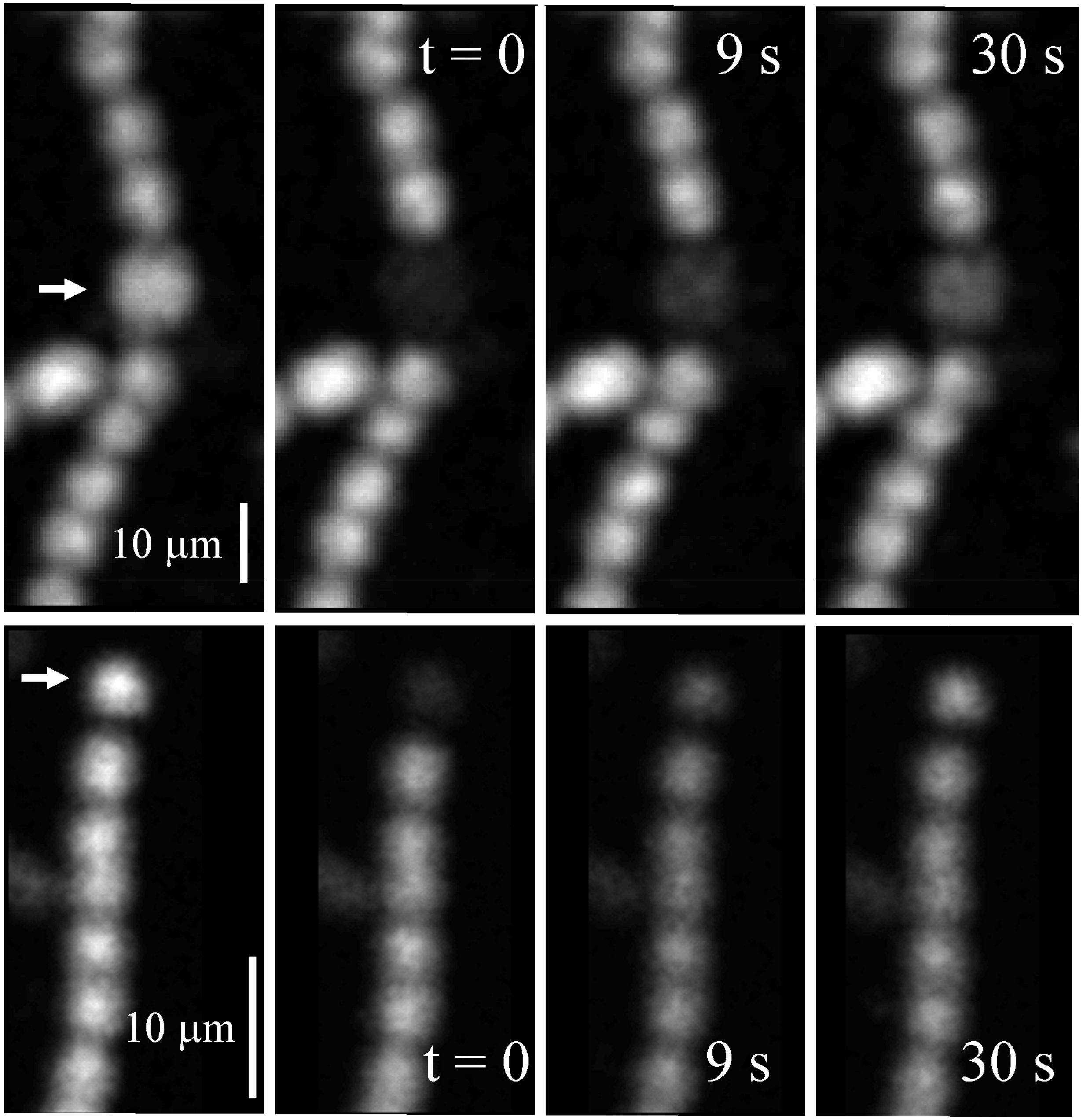
Molecular exchange between vegetative cells and heterocysts is slowed by the presence of cyanophycin polar nodules



Wild-type Δ*cph*A

Anabaena variabilis (ATCC 29413)

Ziegler et al (2001) FEMS Microbiol Lett 196, 13-18

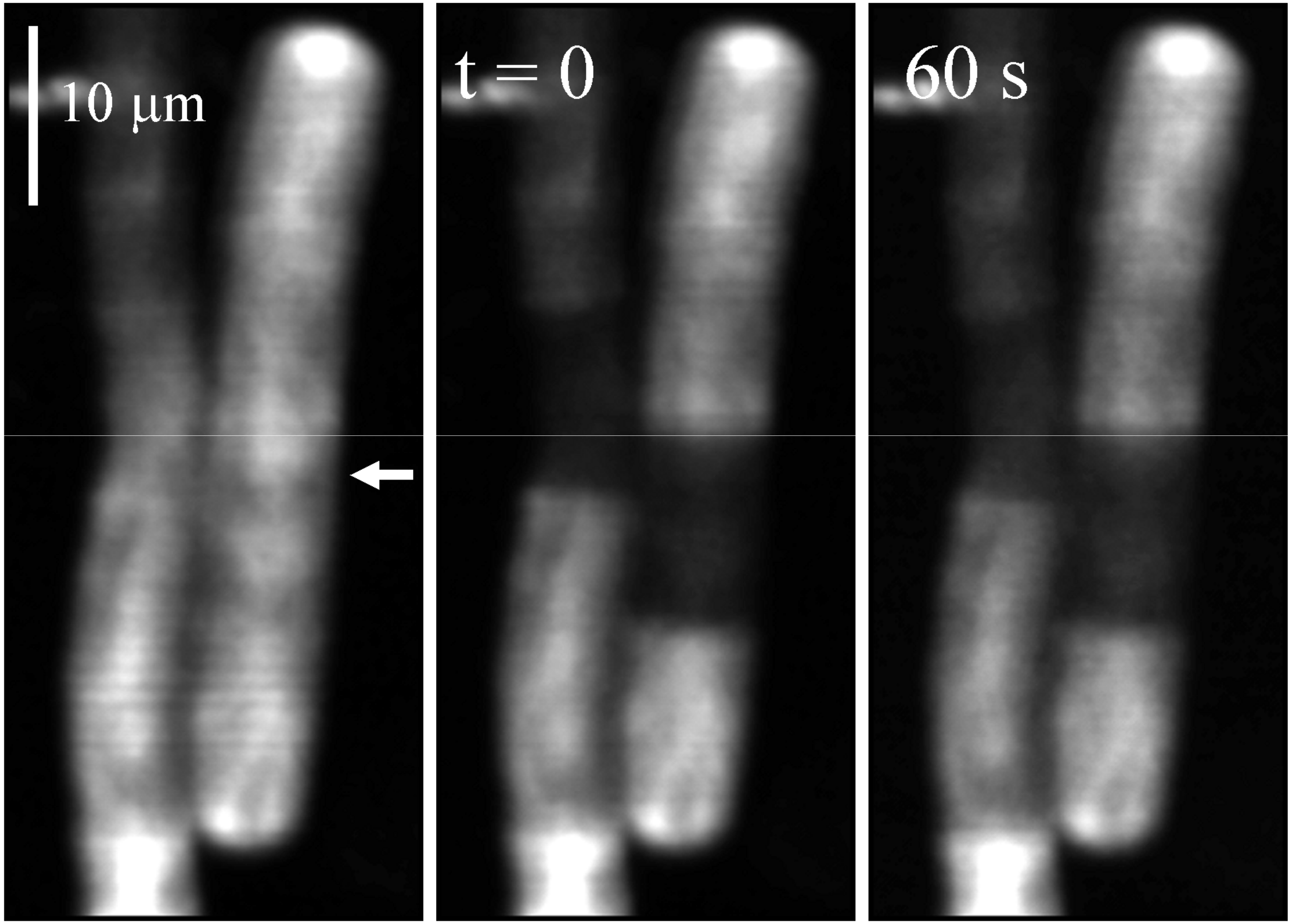


Wild-type
mean E (veg-het)
= 0.020 s^{-1}

Δ*cph*A
mean E (veg-het)
= 0.067 s^{-1}

T-test: P = 0.002

Molecular exchange does *not* occur in *Oscillatoria terebriformis*



What protein machinery is required for molecular exchange?

We are looking for proteins that could form channels from cytoplasm to cytoplasm in neighbouring cells

The channels seem to have similar properties to the gap junctions of animal cells - however there are no obvious connexin orthologs in cyanobacteria

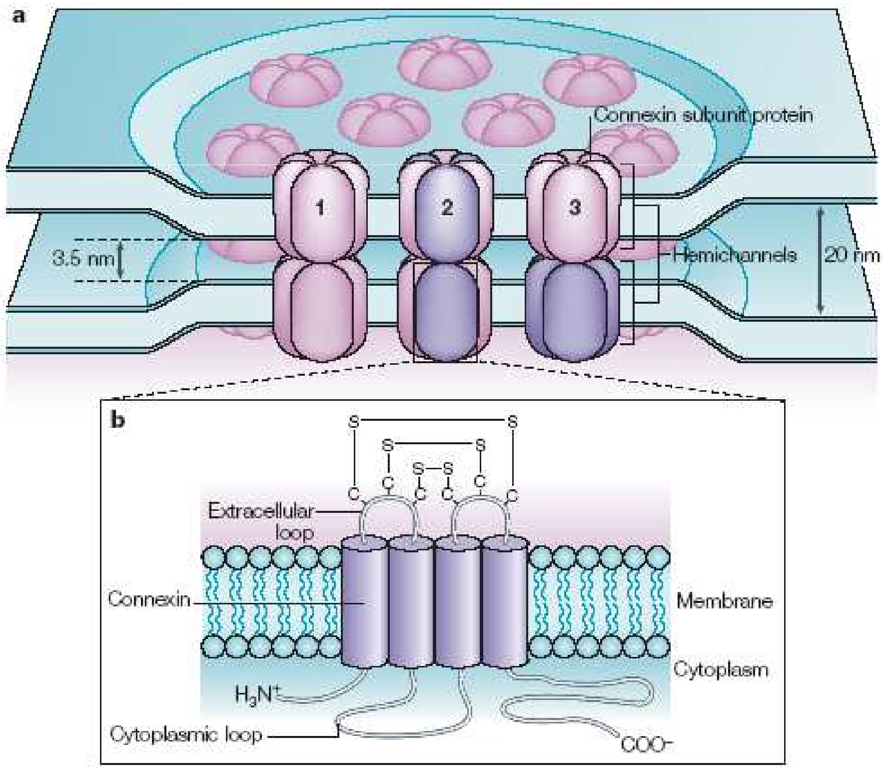
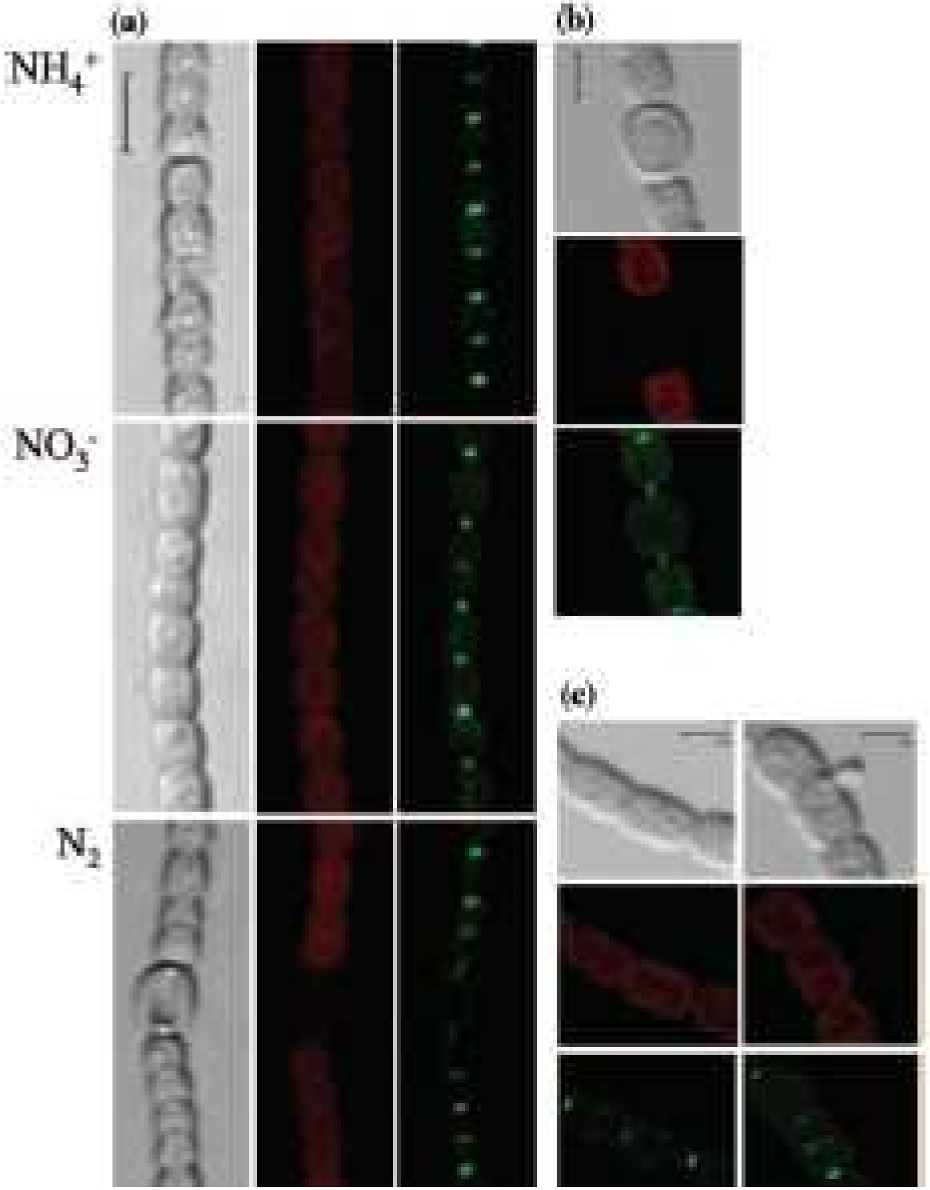


Figure 1 | **Molecular organization and schematic topology of a gap-junctional plaque.**

Söhl et al (2005)
Nature Neuroscience 6, 191-200

SepJ (= FraG), product of ORF *alr2338* in *Anabaena* 7120



SepJ/FraG:

is localised at the cell-cell interface (Veg-Veg **and** Het-Veg junctions)

is conserved only in heterocyst-forming cyanobacteria

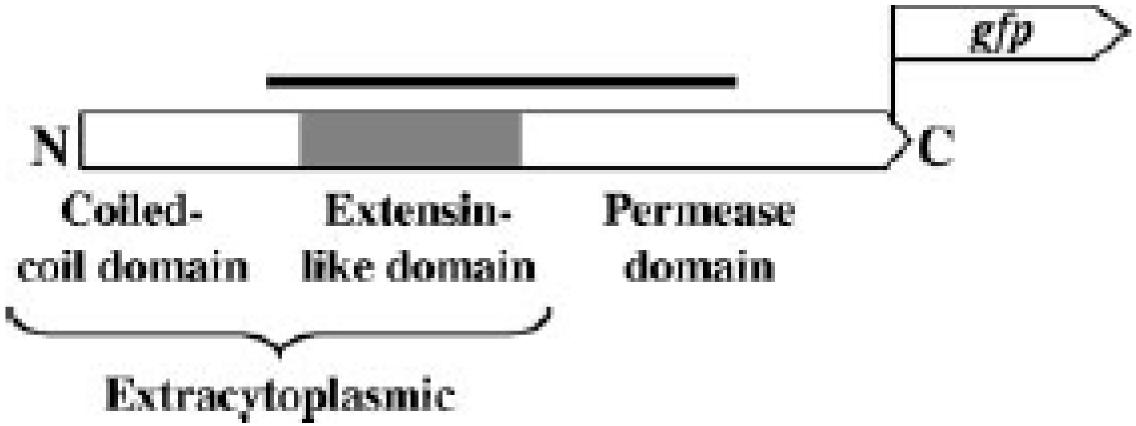
increased transcription after nitrate step-down

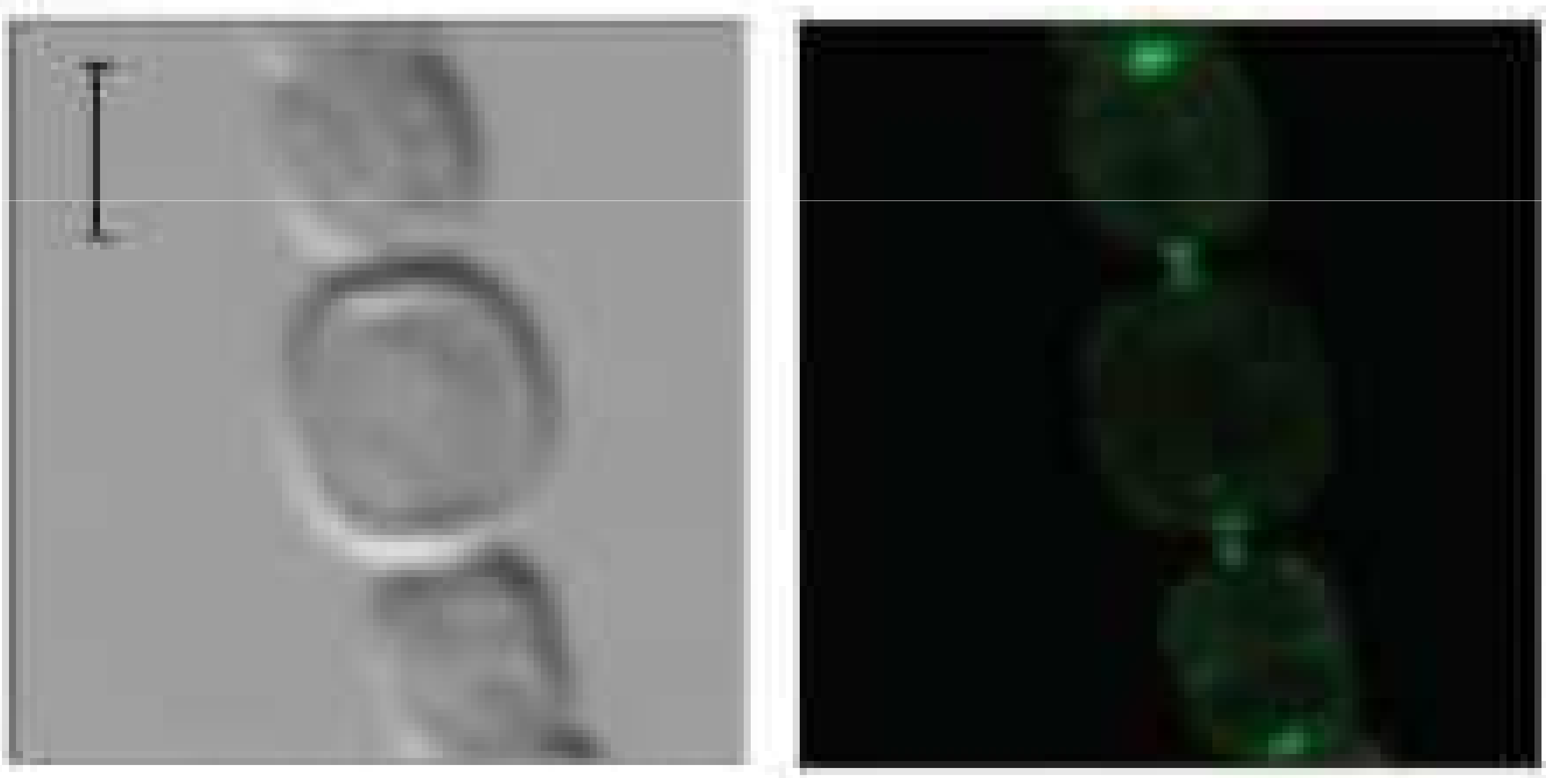
is required for diazotrophic growth and heterocyst formation

has a permease-like (DME) domain!

Flores et al (2007) J. Bact. **189**: 3884-3890

SepJ/FraG





Flores et al (2007) J. Bact. 189, 3884-3890

Mean E values for *Anabaena* PCC7120 (s^{-1})

Nitrate-grown 0.021 ± 0.011

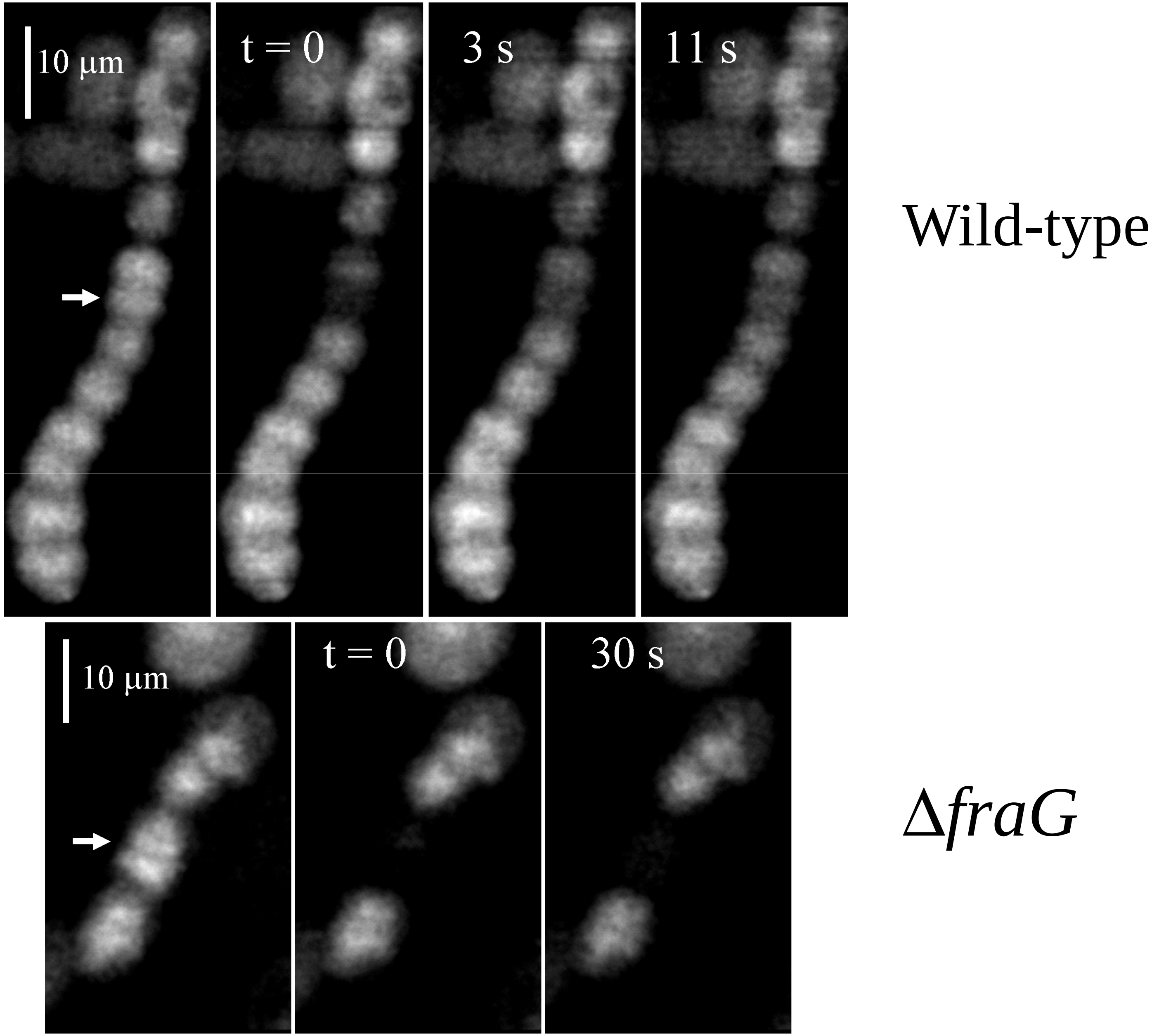
16 hours - nitrate 0.057 ± 0.039

96 hours - nitrate 0.28 ± 0.12 0.035 ± 0.012

Vegetative cells

Heterocysts

Anabaena 7120 after 16 hours minus nitrate



Mean E values for *Anabaena* PCC7120 (s^{-1})

	Wild-type	Δ*fraG*	
Nitrate-grown	0.021 ± 0.011	0.007 ± 0.008	P = 0.004
16 hours - nitrate	0.057 ± 0.039	0.002 ± 0.002	P = 0.004

Tentative conclusions

FraG/SepJ forms channels between cells (probably the “microplasmodesmata” observed by EM)

These channels allow the diffusion of hydrophilic small molecules (at least up to 623 Da) from cytoplasm to cytoplasm

This is the major route for exchange of sugars and amino acids

Up-regulation of channel activity on nitrate step-down leads to faster exchange between vegetative cells (necessary for amino acid supply from the heterocysts)

Molecular exchange with heterocysts is slower - necessary to keep the cytoplasm microaerobic. Slower exchange partly due to polar nodules

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Δ*cphA*
Wolfgang Lockau (Berlin)

Oscillatoria
Richard Castenholz (Oregon)

Encouragement and advice
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Preliminary work on CellTracker
Rasmi Pillai (London)
Mary Sarcina (London)

Paid for equipment etc (though **not** intended for **this** project....)