Engineering and Physical Sciences Research Council

The Clostridial Cell Biology Group
The University of Sheffield

Insights into spore germination: Live spore imaging in *C. sporogenes*

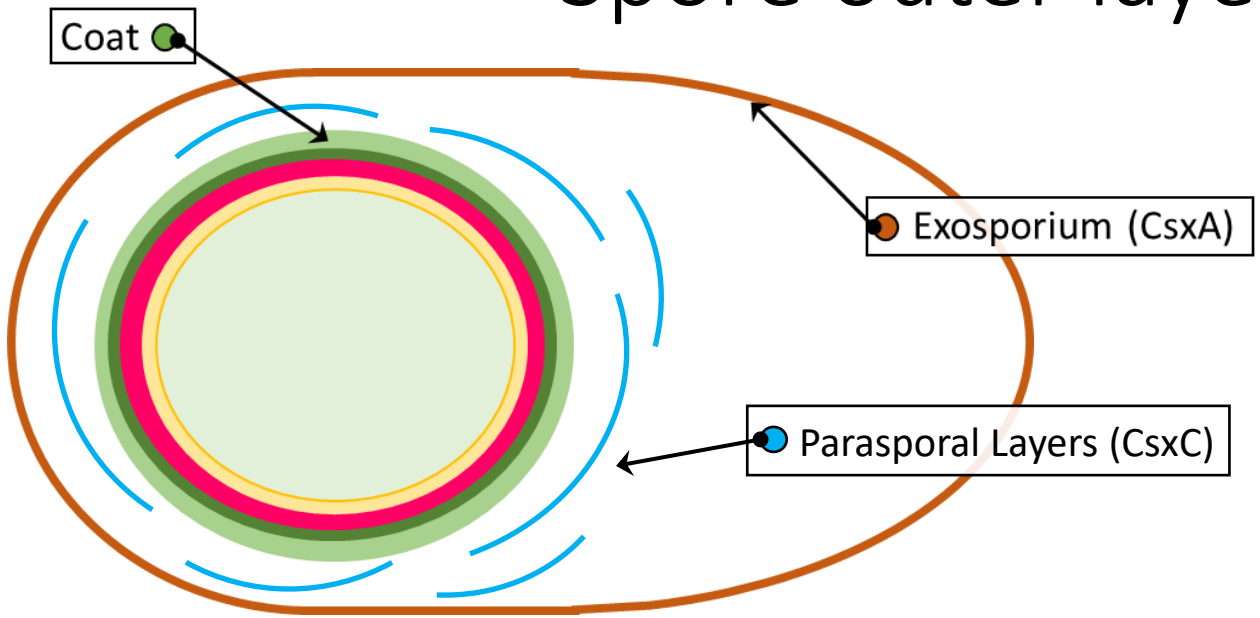
Anne Williams

Hannah Fisher, Robert Fagan, Per Bullough, William M Durham

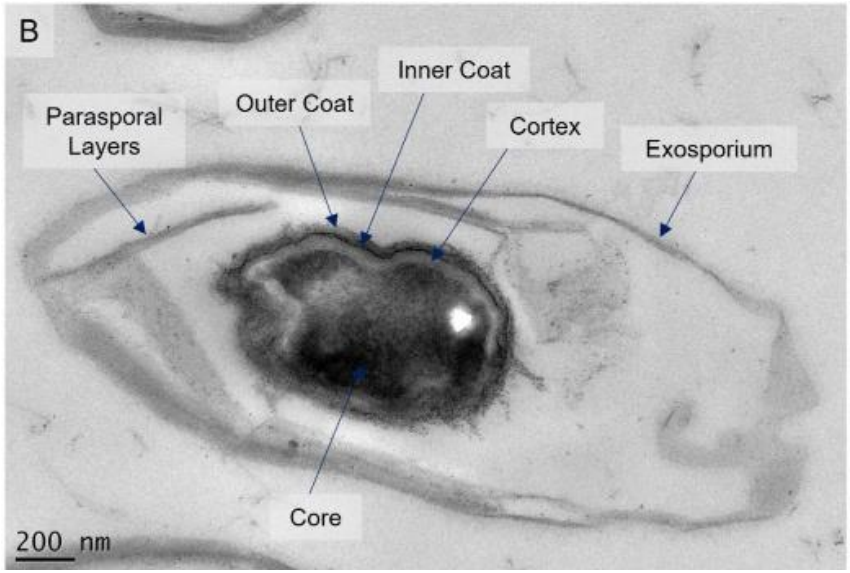
University of Sheffield

MICROBIAL PHYSICS Group
The University of Sheffield

Spore outer layer map



CsxB

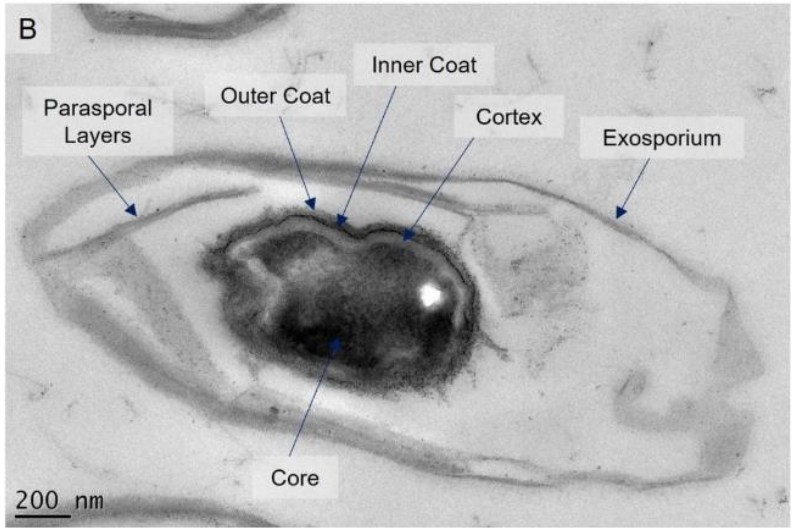


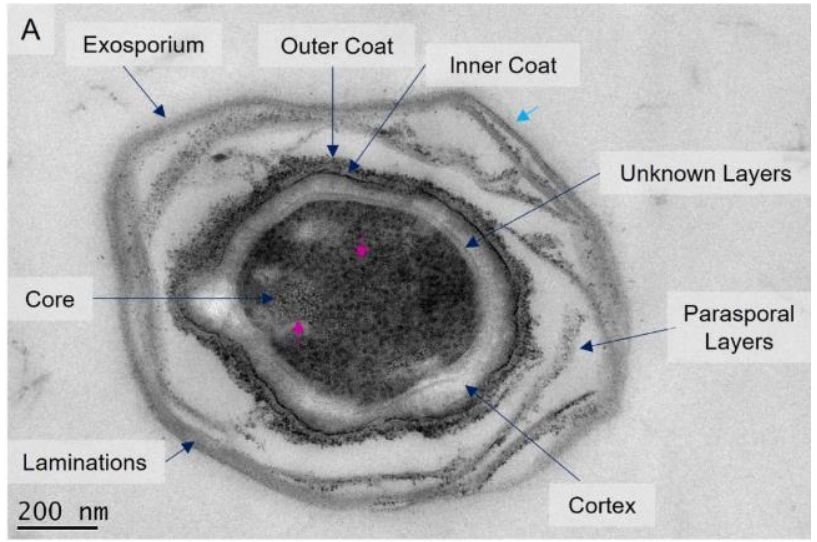
CsxC

Fisher, 2022 (Doctoral thesis)

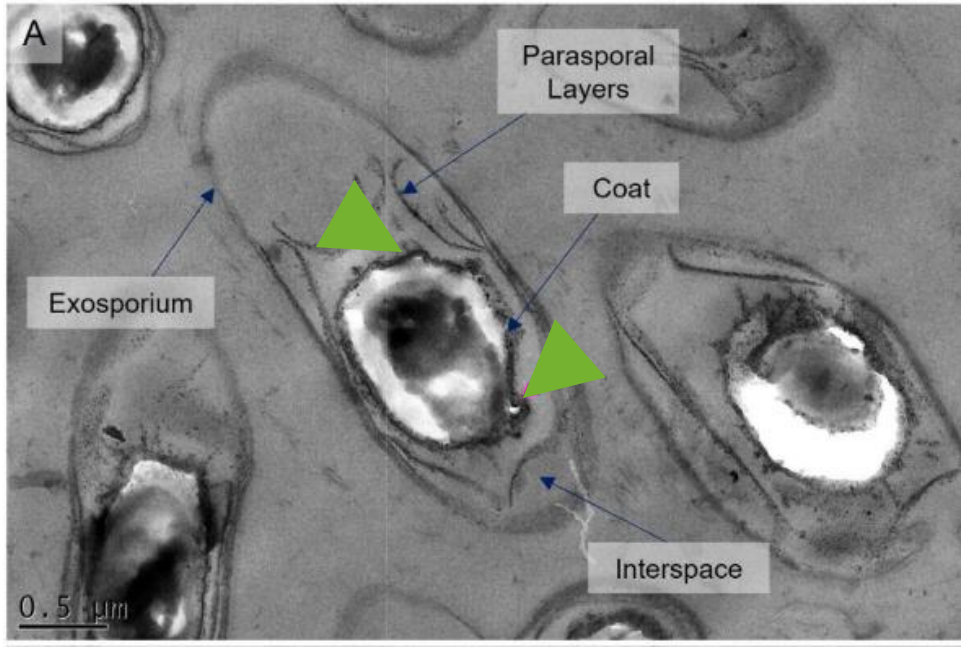
CsxB: where am I?

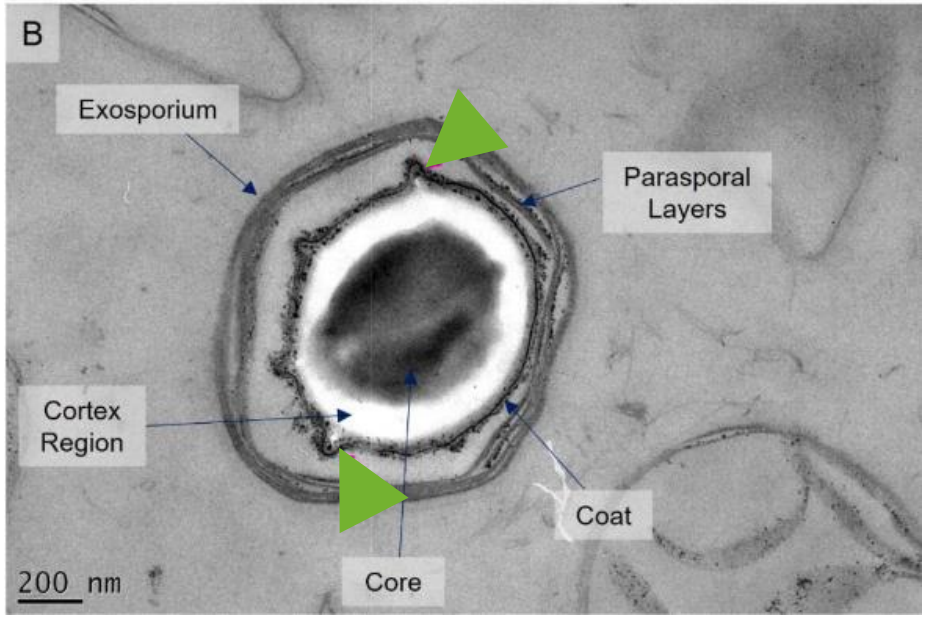
WT:





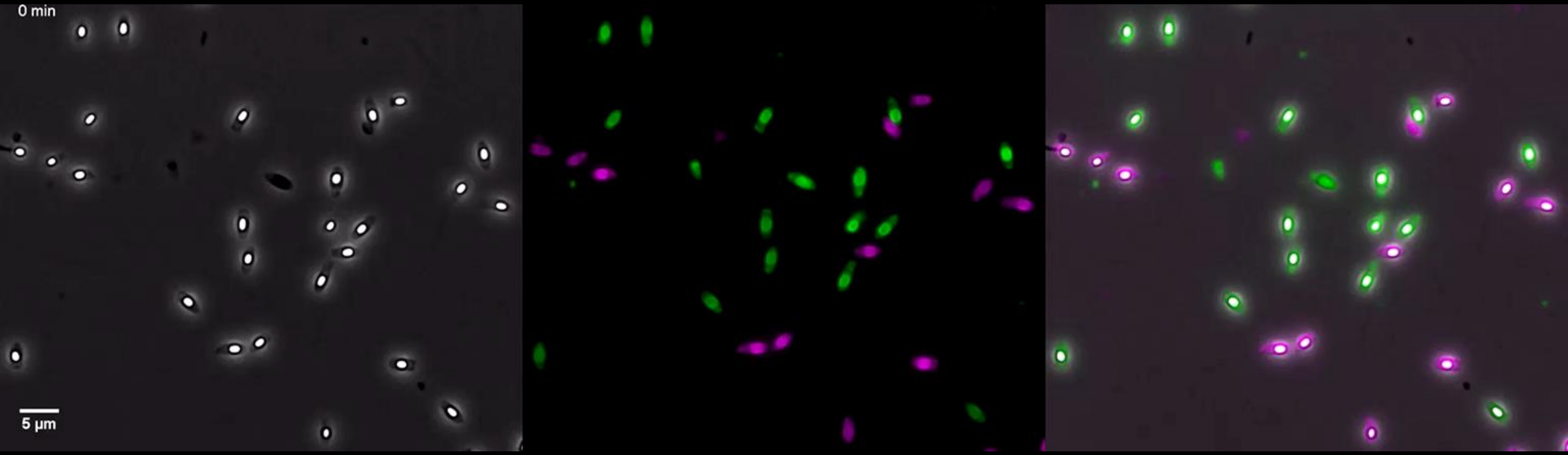
ΔcsxB:





Hannah Fisher, 2022 (Doctoral thesis)

Imaging anaerobes in co-culture



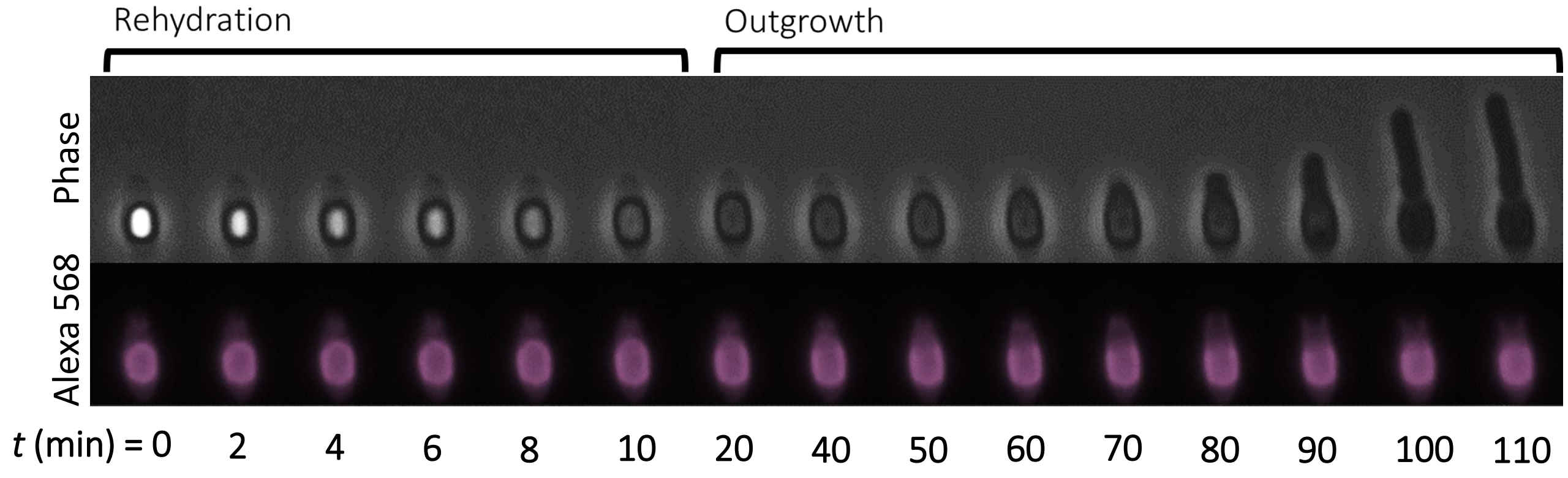
Phase

WT - Alexa 568

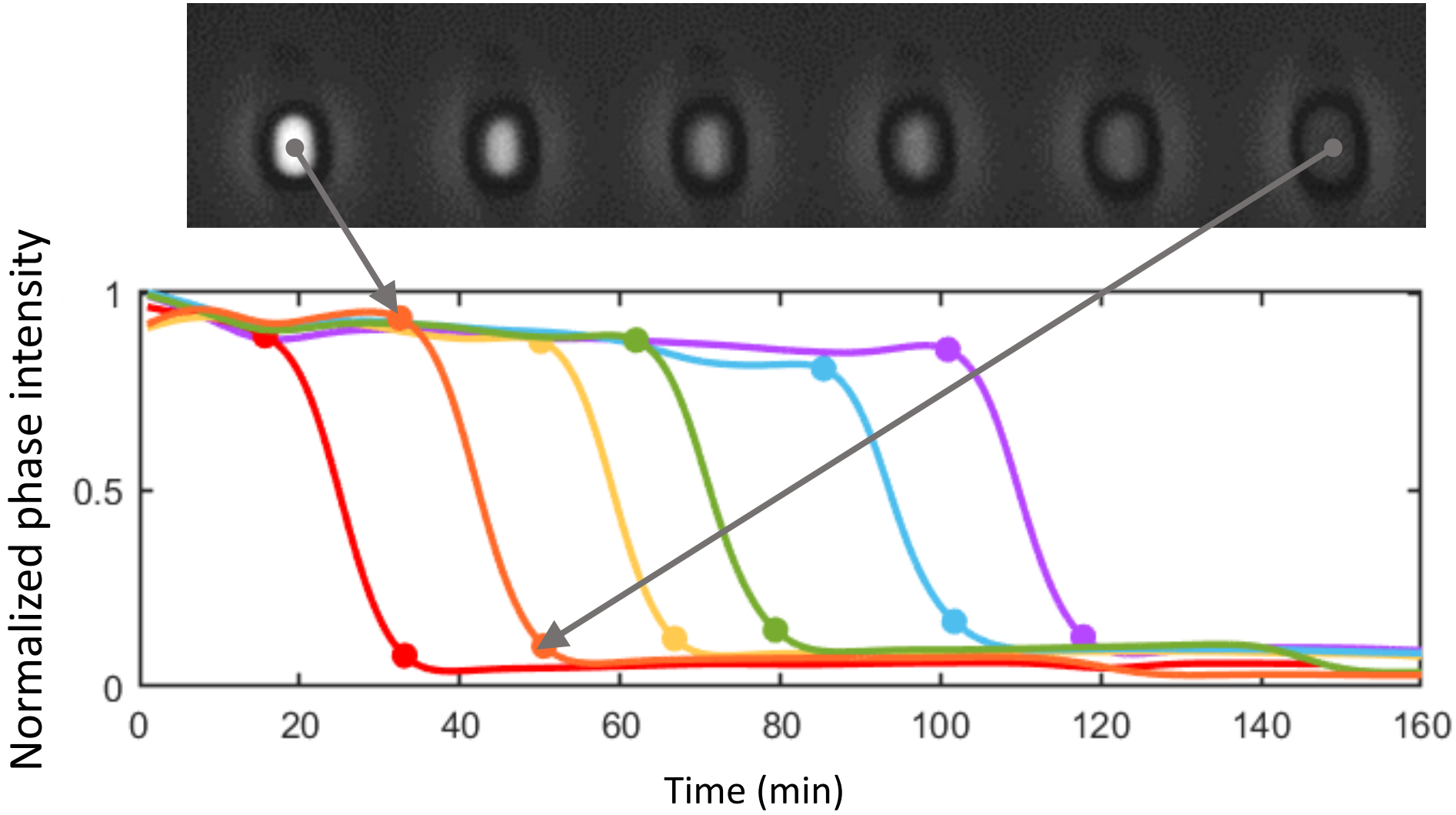
ΔcsxB - Alexa 488

Merged

Quantifiable Stages of Germination



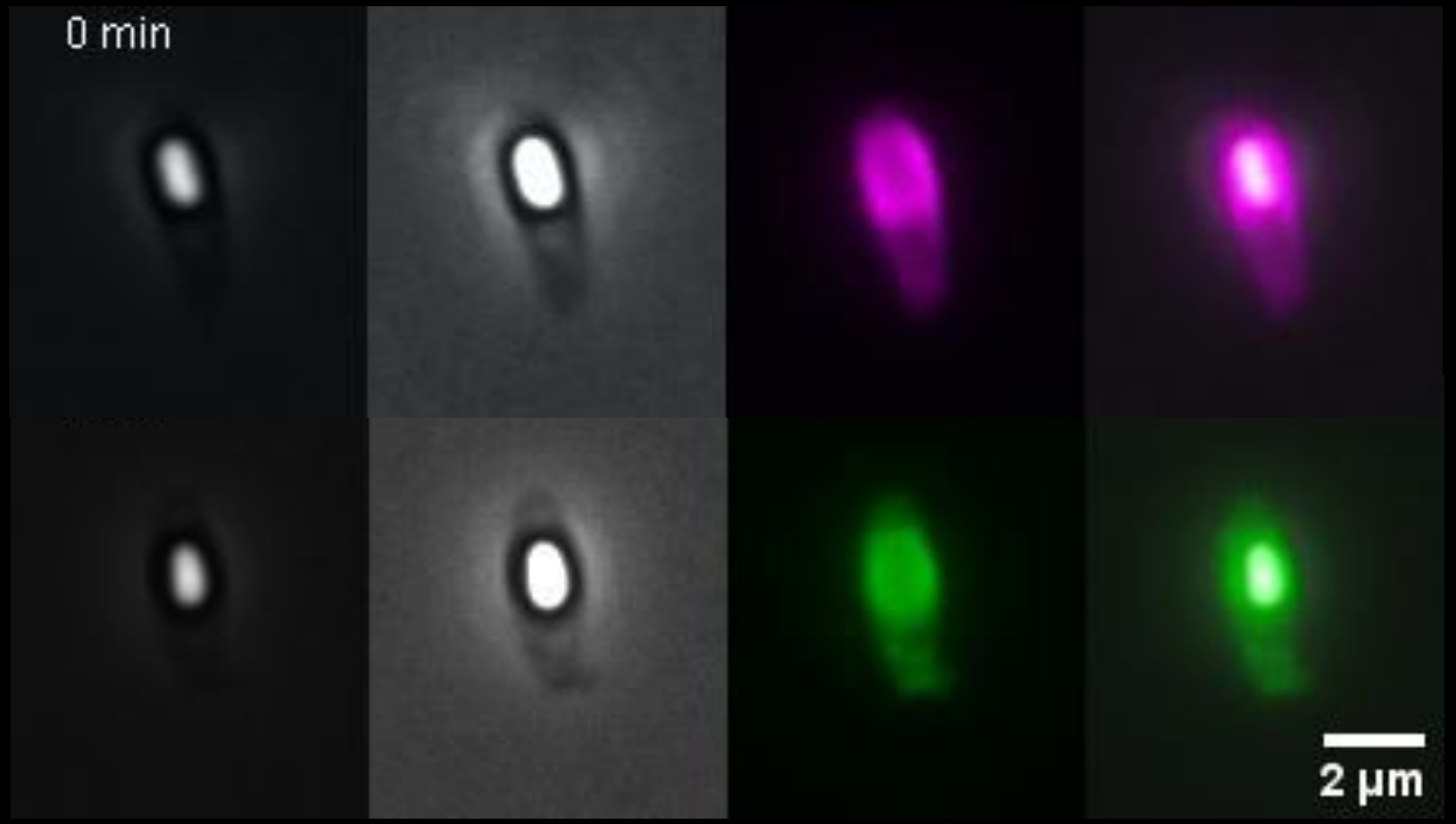
Resolving rehydration timing using core intensities



Δ*csxB* shows a distinct phenotype

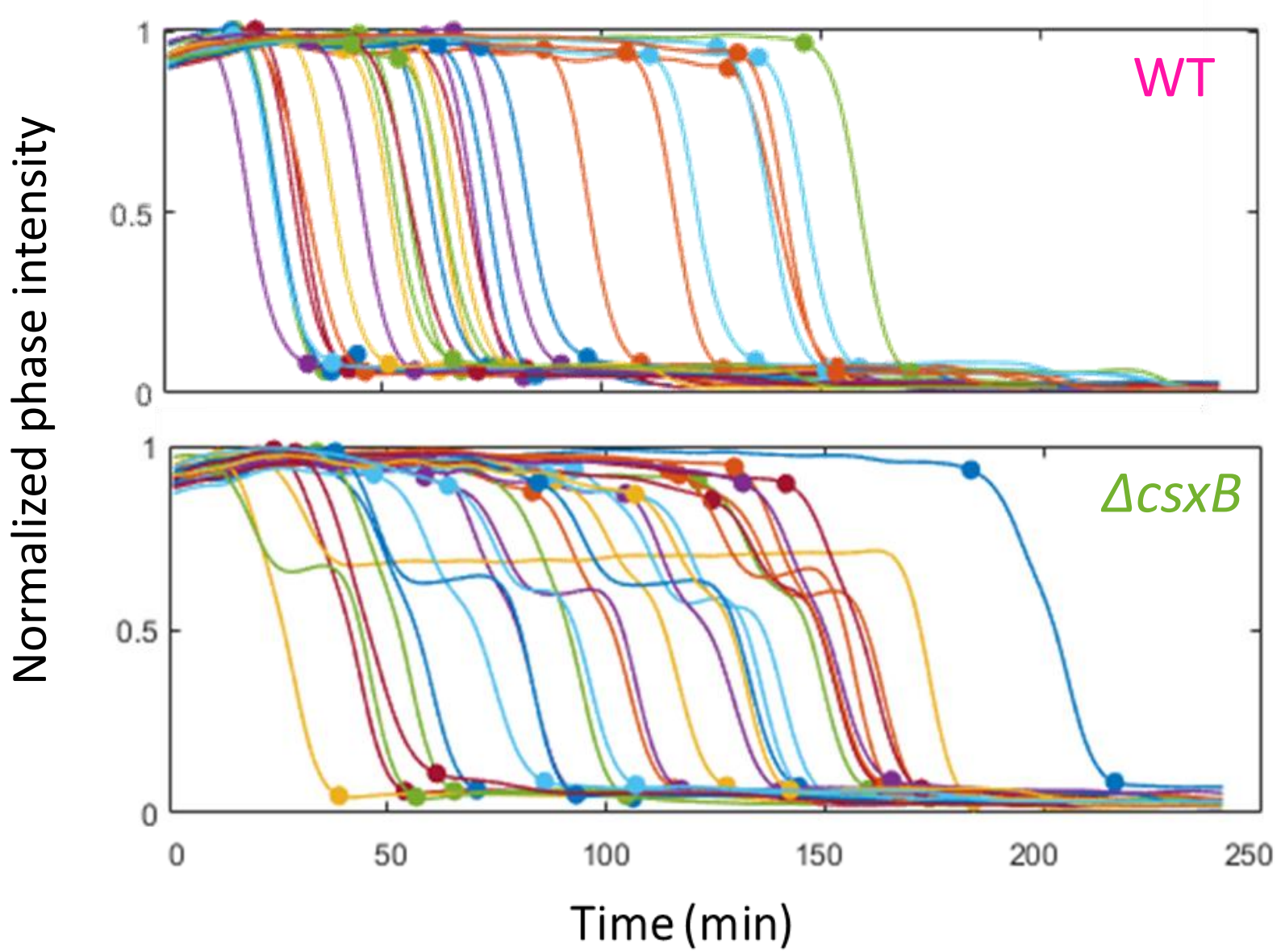
WT

Δ*csxB*



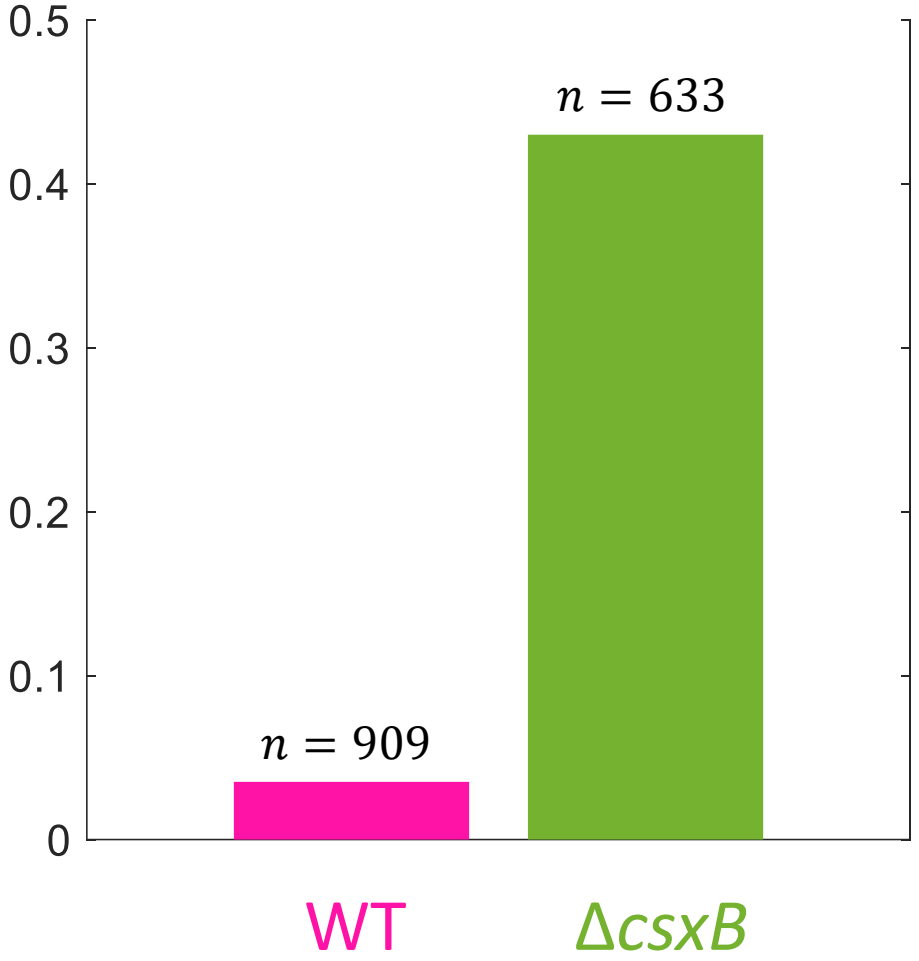
Phase (raw) Phase (high-contrast) Alexa 568/488 Merged

Δ*csxB* exhibits two-stage rehydration

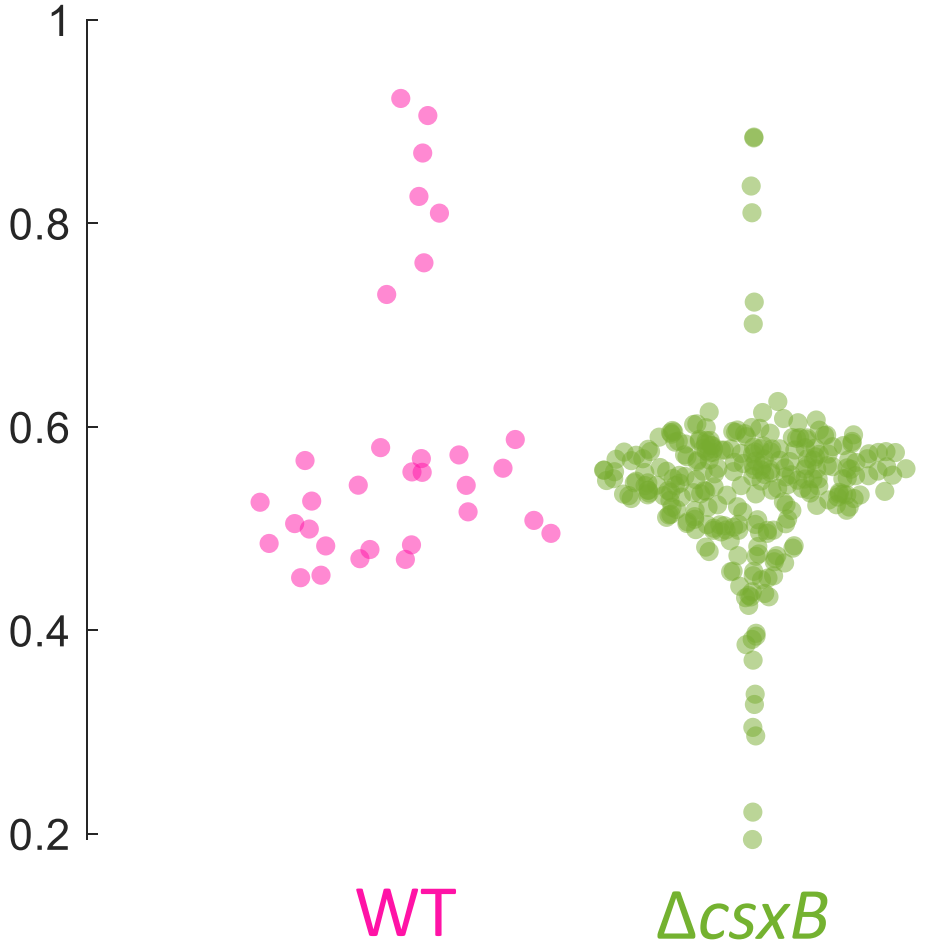


Δ*csxB* exhibits two-stage rehydration

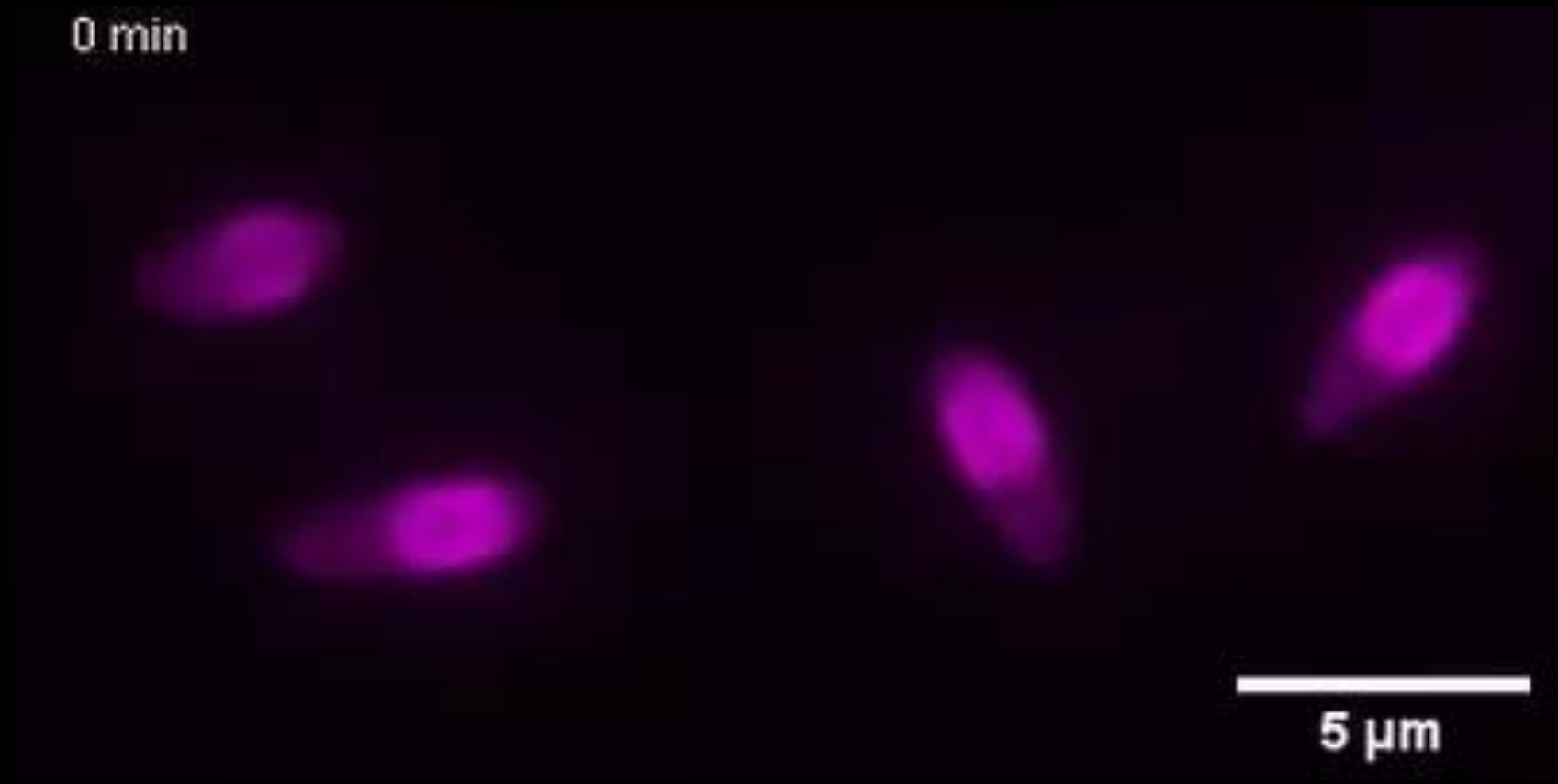
Fraction of spores with a stall in rehydration



Normalized Intensity at the time of the stall

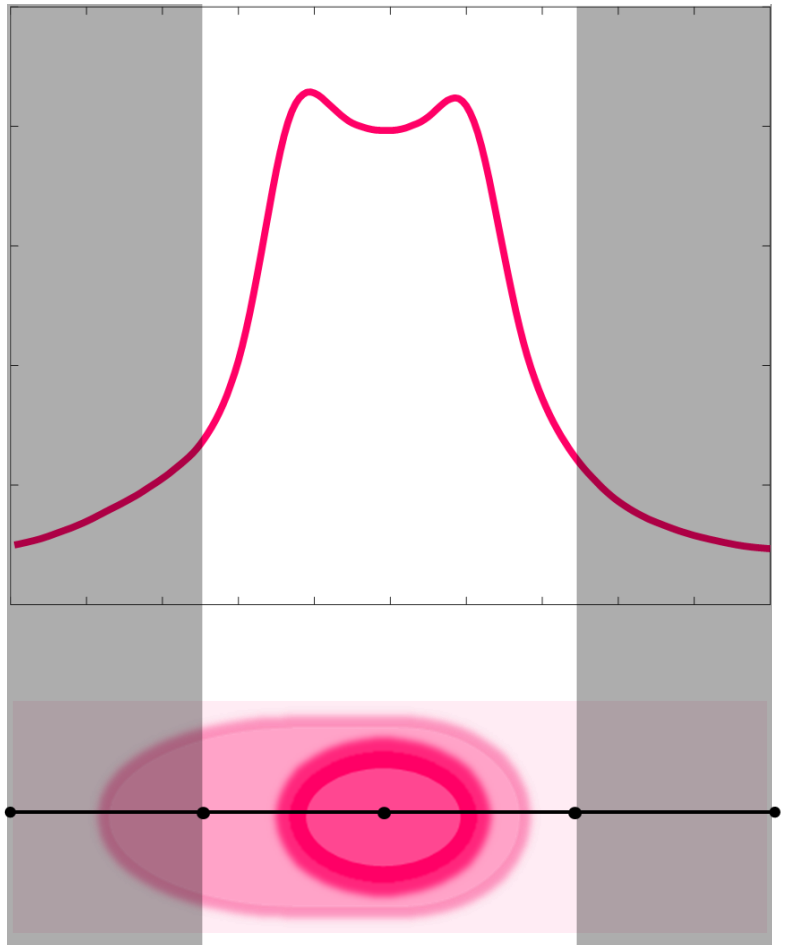


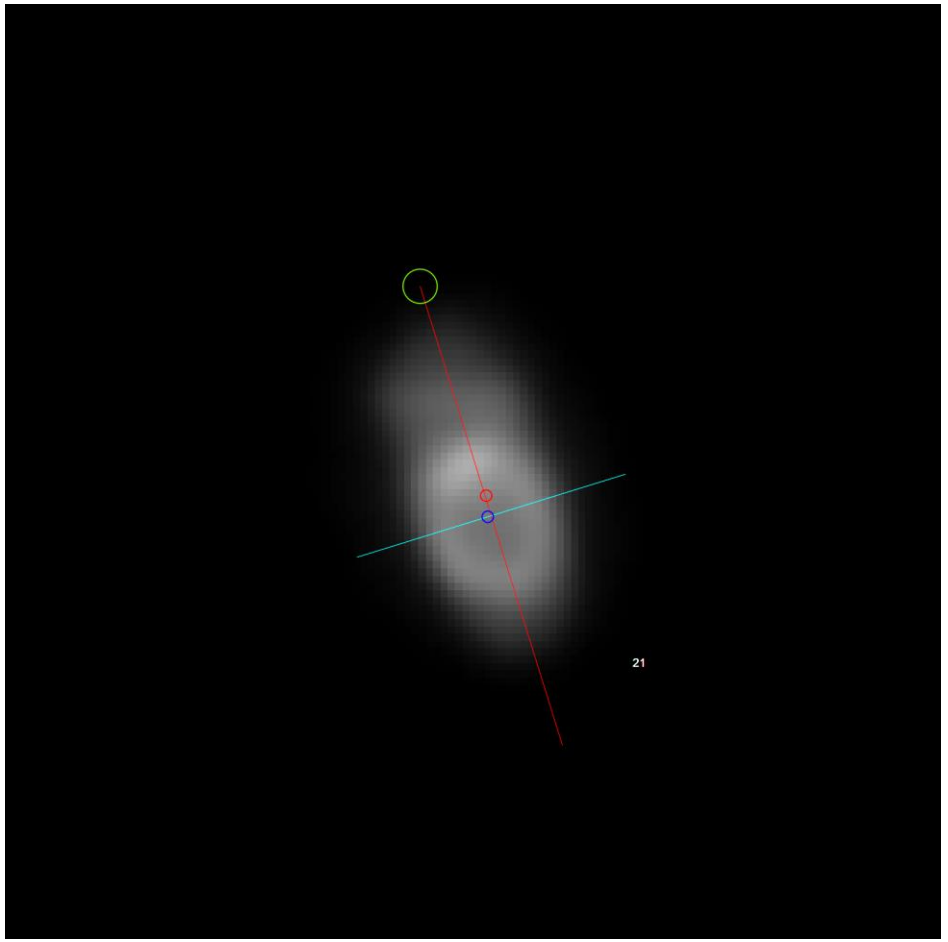
Utilizing fluorescence to visualize protein layers



Exosporium polarity

Finding the distal pole of the exosporium

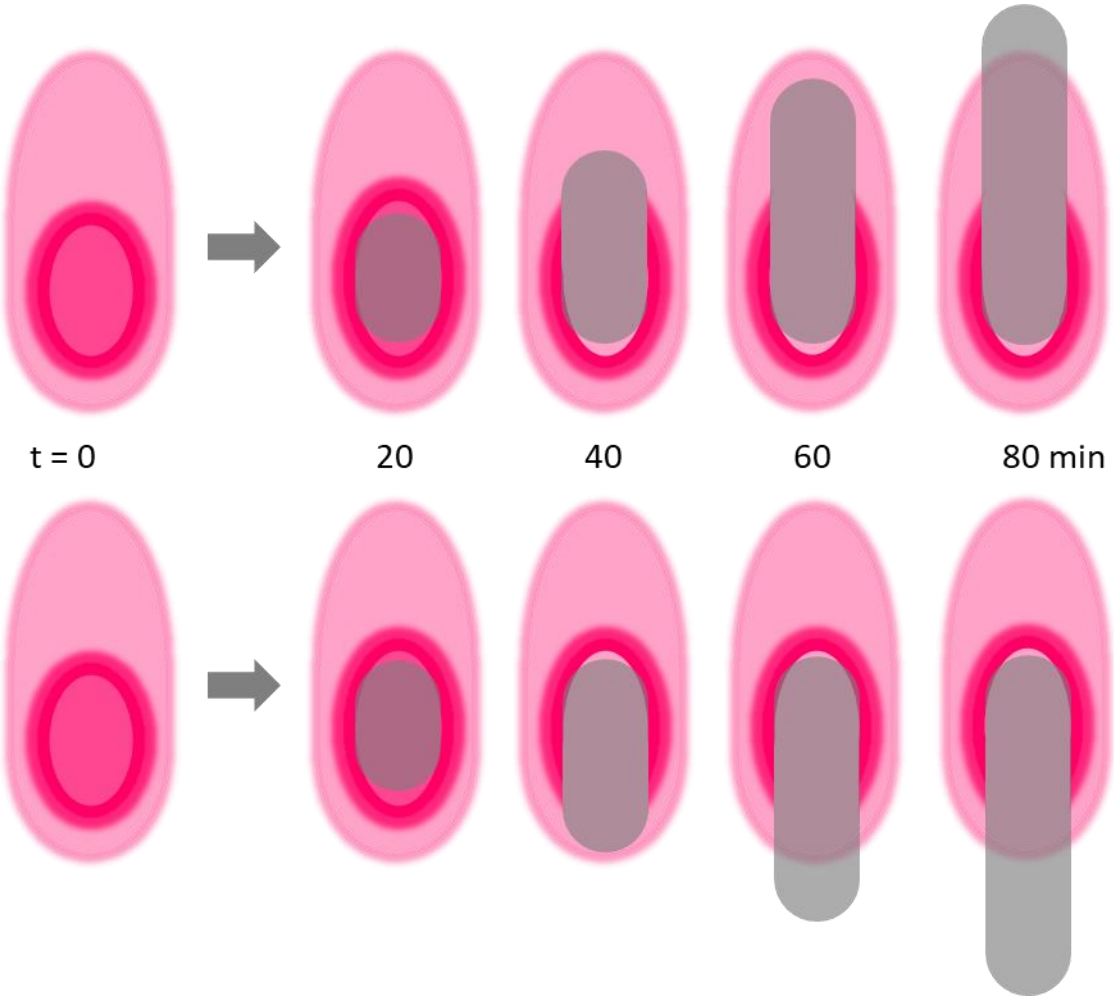




Exosporium polarity

Emergence from

distal pole

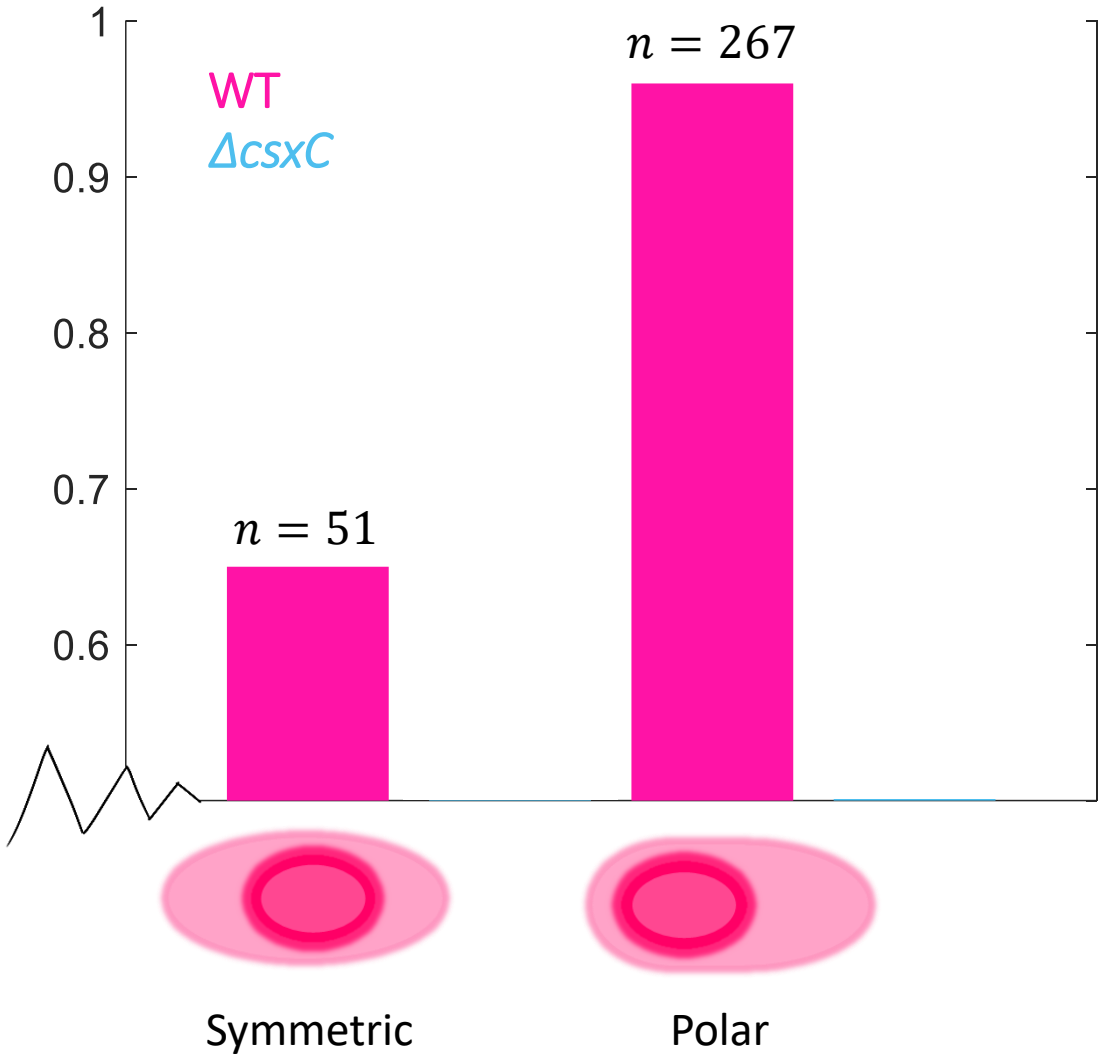


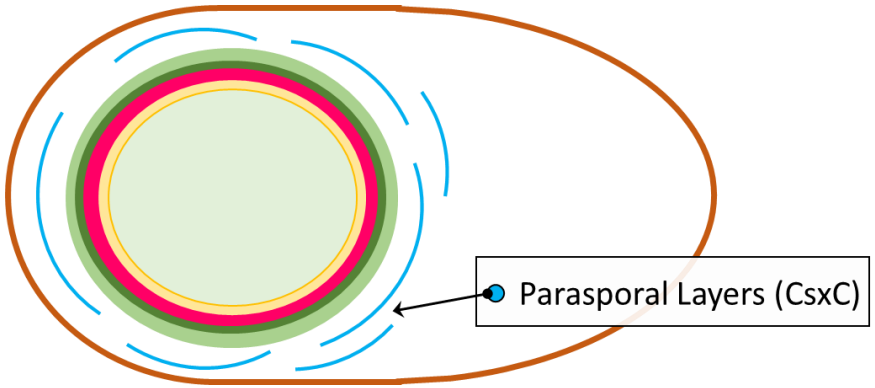
Emergence from

proximal pole

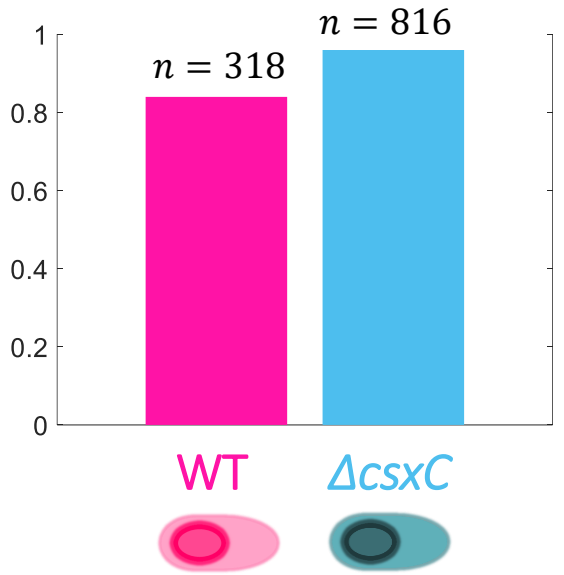
Vegetative cells emerge from the distal pole

Fraction of cells emerged from the distal pole

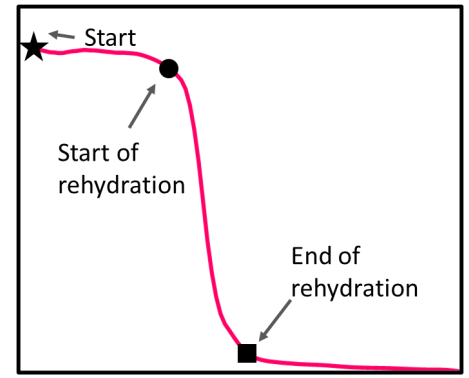


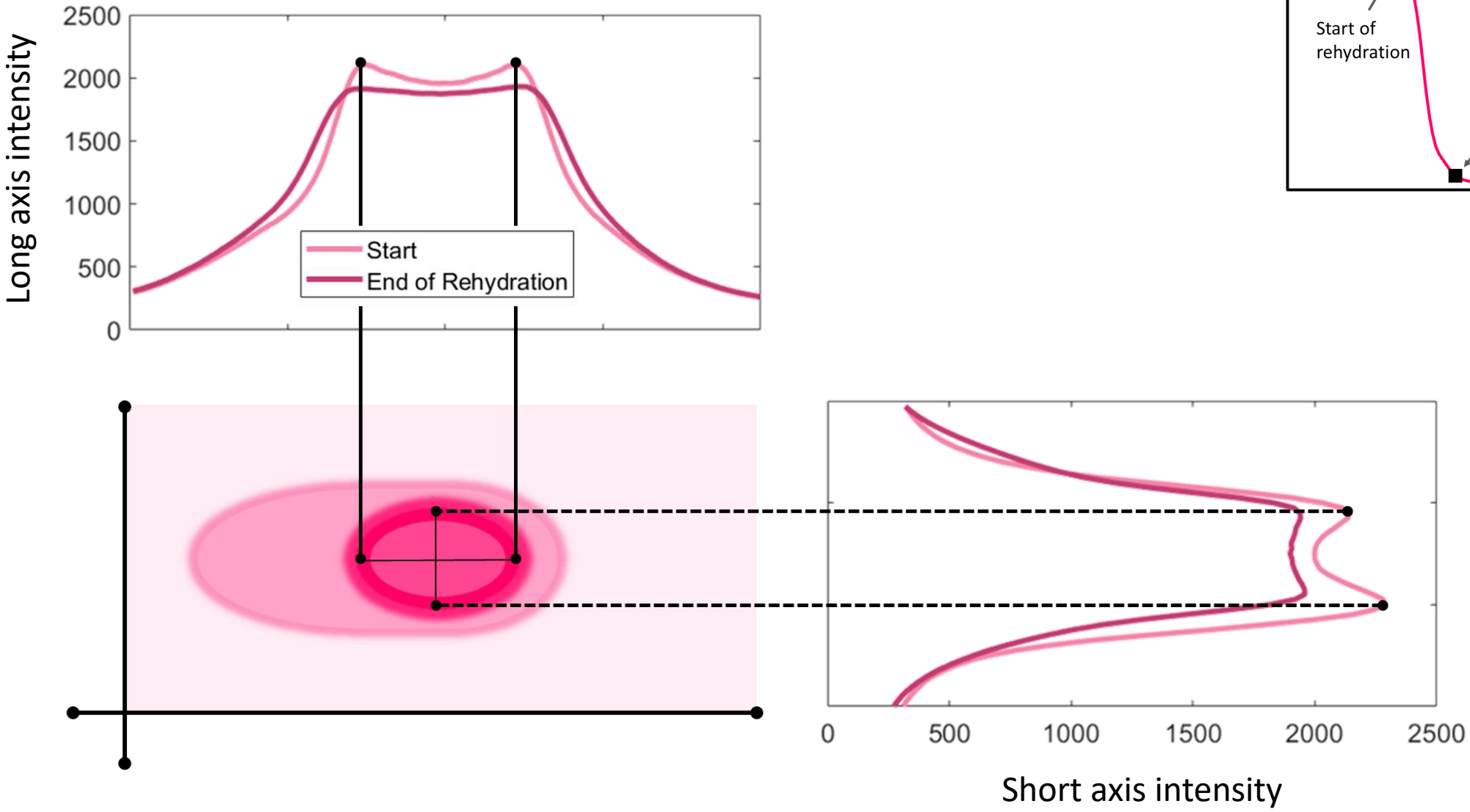


Fraction of polar cells

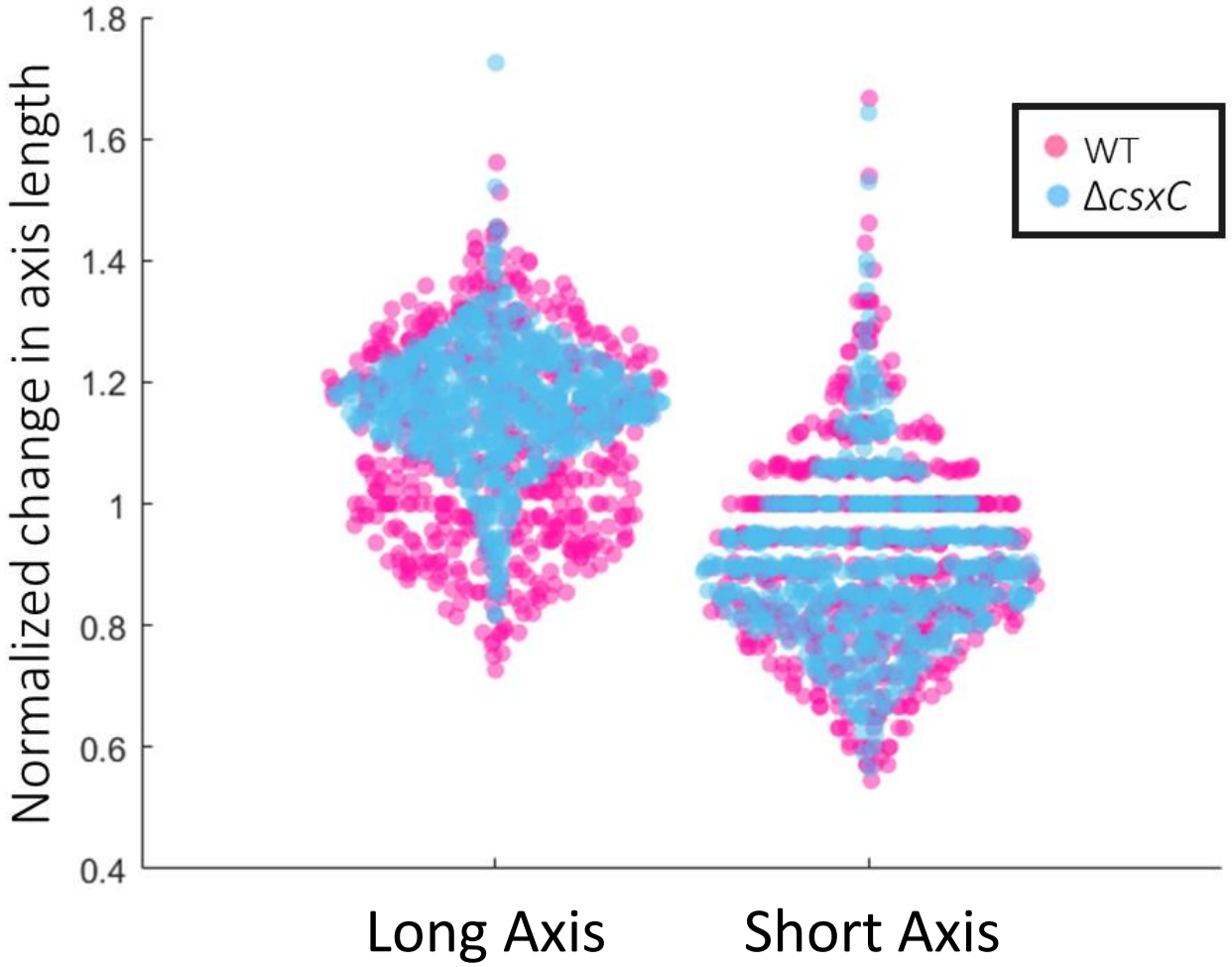


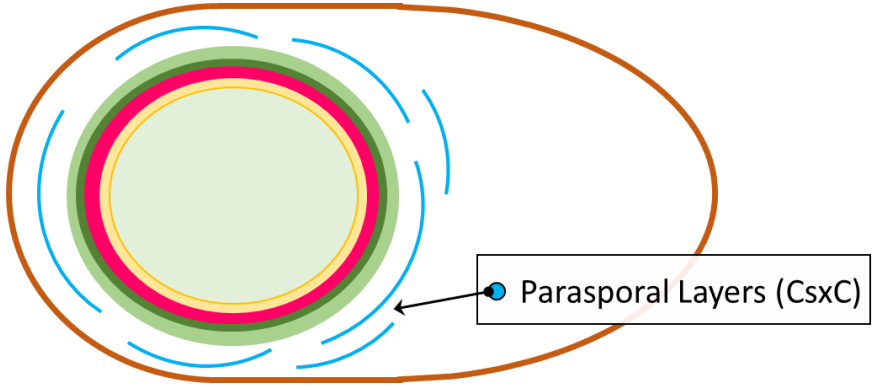
Measuring Coat Expansion

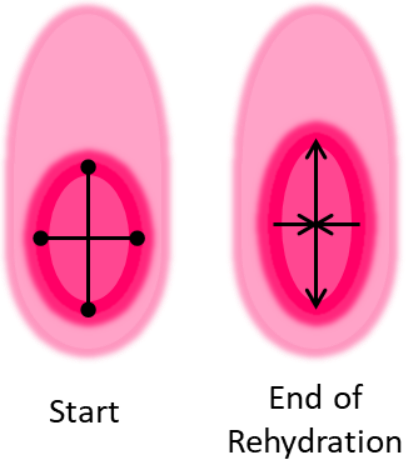


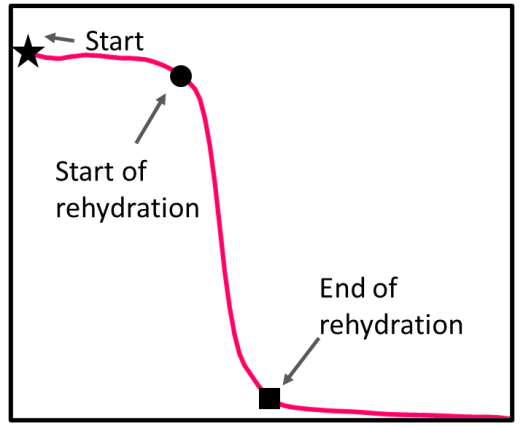


ΔcsxC always exhibits coat expansion in the long axis









Summary!

- We have developed a platform to image **co-cultures** of **anaerobes** to quantitatively resolve phenotypes
- Δ*csxB* spores exhibit **two-stage rehydration**
- WT cells **emerge** from the **distal pole** of the exosporium
- Δ*csxC* spores exhibit a robust **expansion** along their long axis **compared to WT**

Thank you!

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Jess Buddle
Anirudh Jakhmola
Lilia Hodge
Becky Menday
Conrad Mcdonnell

@anneswilliams.bsky.social

aswilliams2@sheffield.ac.uk

All spores in this work are prepared within a Don Whitley Scientific anaerobic workstation.

don whitley scientific
culturing innovation

UKRI Engineering and Physical Sciences Research Council

HFSP

The Clostridial Cell Biology Group
@RobFagan

MICROBIAL PHYSICS Group
The University of Sheffield

Exosporium polarity: *ΔcsxC*

Finding the distal pole of the exosporium