Quantifying the effect of substrate composition on intra-colony channel morphology in *E. coli* biofilms using a custom-made open-source image analysis pipeline

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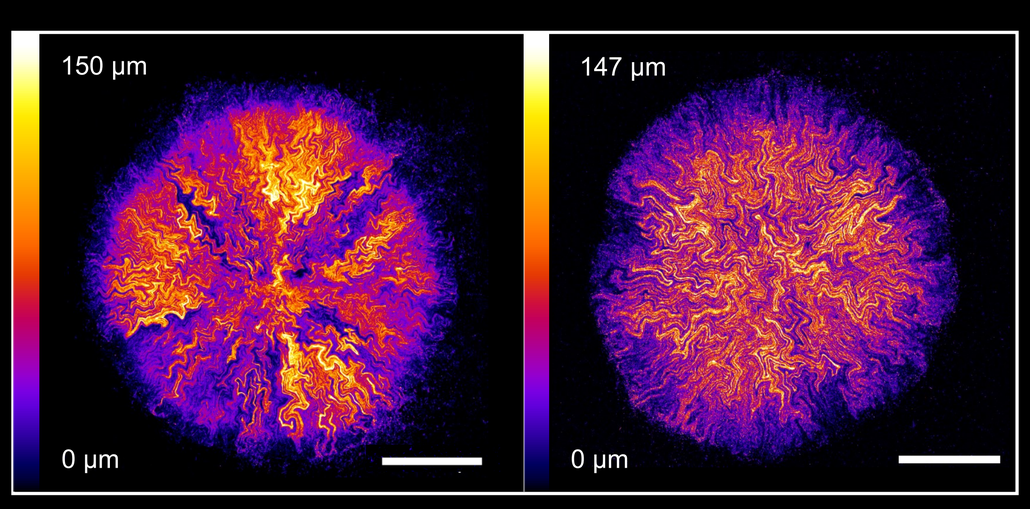
Introduction

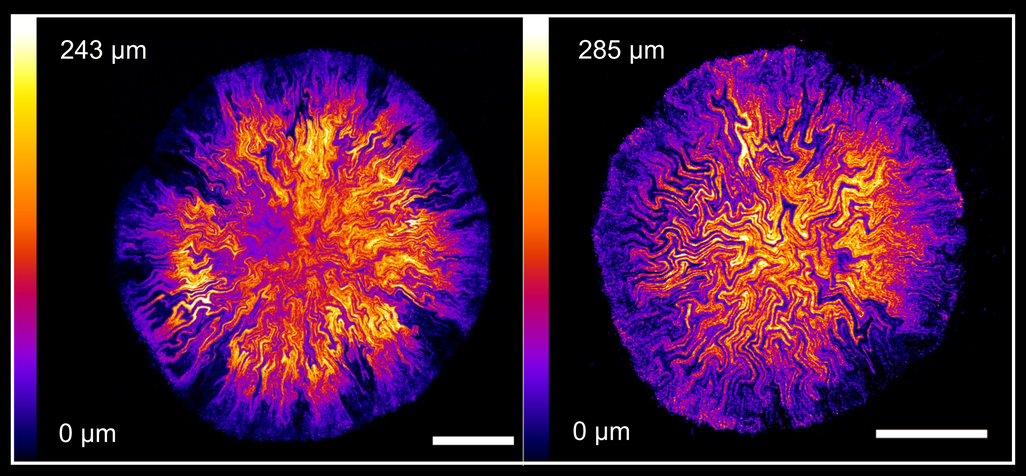
- **Networks of intra-colony channels are involved in nutrient transport inside mature *E. coli* biofilms**
- **We hypothesise that nutrient availability and substrate composition affect biofilm morphology and channel architecture**

Results

- **Nutrient availability affects channel morphology at the mesoscale: channels forming on nutrient-rich substrates have a more complex fractal structure**



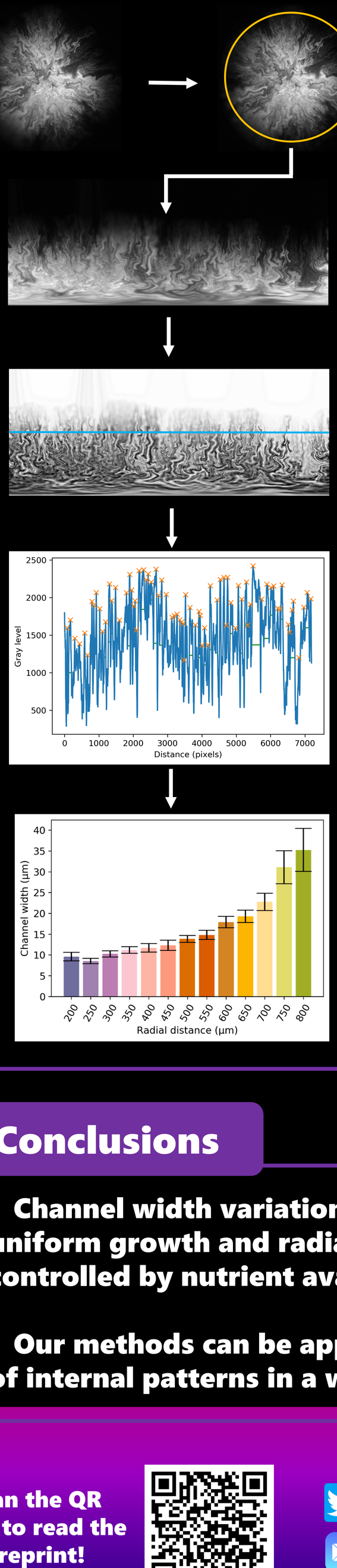
Increasing carbon concentration



Increasing nitrogen concentration

- **Channel width increases non-linearly with radial distance from the centre under all nutrient concentrations**

Methods



1. **Widefield epi-fluorescence images of biofilms acquired with the Mesolens**
2. **Polar transformer plugin used to convert image from polar to cartesian**
3. **Contrast enhancement and colour inversion**
4. **Line profiles taken at different radial distances from the colony centre, then imported into Python**
5. **Python script used to identify peaks and calculate their full width at half-maximum (FWHM)**
6. **FWHM converted to channel width using polar geometry**

Conclusions

- **Channel width variation could be a result of non-uniform growth and radial expansion, which are controlled by nutrient availability**
- **Our methods can be applied to the quantification of internal patterns in a wide range of biofilms**

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