

# Fluorescence hyperspectral signatures of *Pseudomonas* challenge and flg22 in *Arabidopsis*

A FAIR reanalysis of the 2016 UW-Madison experiment

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[PENDING: date / venue]

# The question

- Can we **see** a plant responding to bacteria before symptoms appear?
- Plants perceive flagellin (flg22) through the receptor **FLS2**; *fls2* mutants cannot.
- **CML24** (TCH2) is a  $\text{Ca}^{2+}$  sensor; *cm124-4* is impaired in pattern-induced NO signalling.
- Chlorophyll fluorescence changes with photosynthesis and chlorophyll content, so a **whole emission spectrum per pixel** might carry an early signature.

**Key control:** a genuine flg22 *perception* signature must vanish in *fls2*.

# The 2016 experiment

- MacroPhor fluorescence hyperspectral imager (Middleton Spectral Vision), Gilroy Lab
- 488 nm excitation, 500–800 nm emission, 324 bands
- 5 genotypes per plate: Col-0, *fls2-17*, *fls2-101*, *cml24-2*, *cml24-4*
- Mock, flg22 or *P. syringae*; 4 plates each
- Imaged before treatment, then 1, 2, 3, 24 and 48 h
- Independent replicate (Nov 2016, 405 nm excitation)

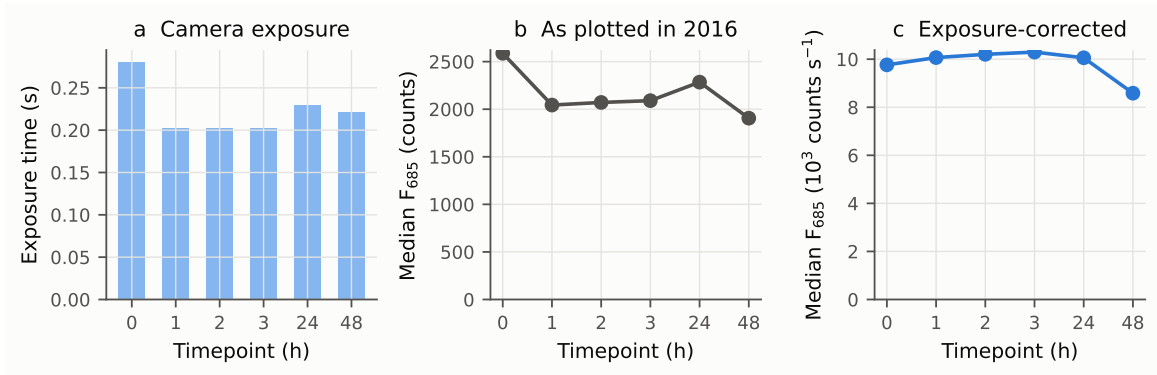
## Recovered from the drive

7,752 files, 396 GB

400 image cubes

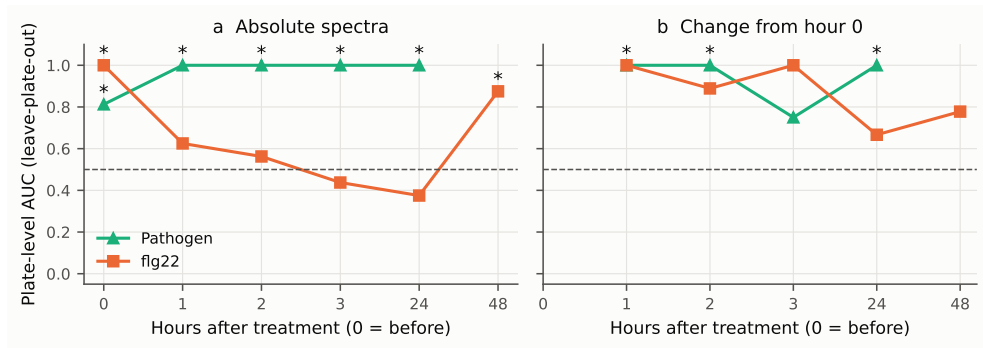
2,486 unique hand-drawn seedling spectra  
every file name parsed, every cube hashed

## Data problem 1: exposure time changed between timepoints



The 2016 graphs' 21% intensity fall after treatment is a camera setting: after dividing by exposure, 1–3 h Control values lie within 4% of hour 0.

## Data problem 2: plates differed *before* treatment

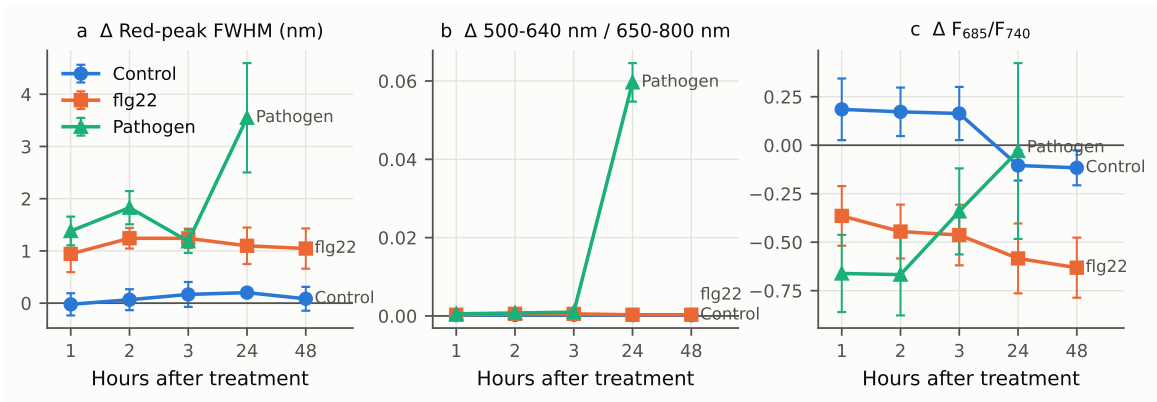


(a) At hour 0, before any treatment, flg22 and Control plates are perfectly separable (AUC 1.00). Only change from each plate's own baseline is evidence (b).

## Data problem 3: some 2016 exports kept the dark level

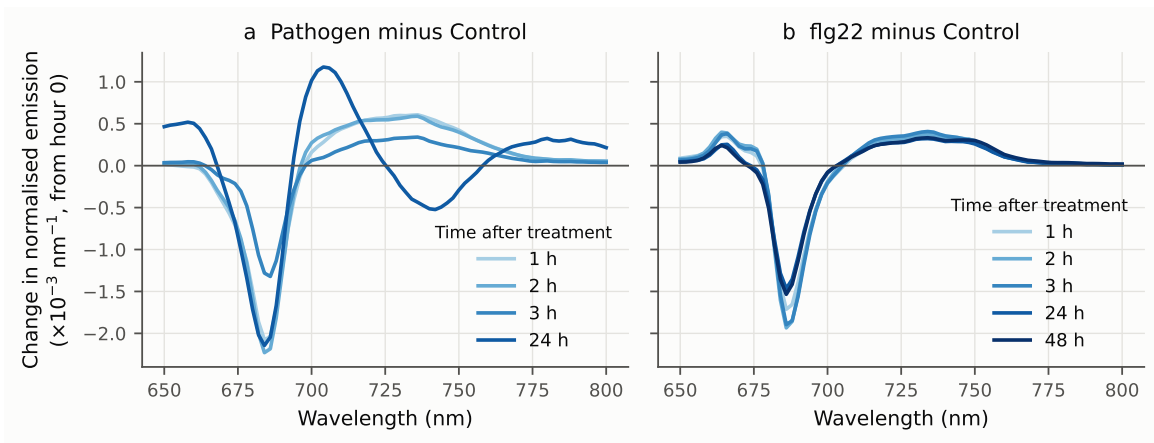
- 83 hand-drawn spectra (all on Pathogen plates, 3 h and 24 h) were saved without dark subtraction: a flat 109-count offset
- Uncorrected, it looks like broad “autofluorescence” below 640 nm
- Nothing fluoresces below the 488 nm excitation, so each spectrum’s 420–470 nm mean is its own offset: subtracted before any analysis

## A pathogen signature from 1 hour



Red emission peak broadens from 1 h (+1.40 nm,  $q = 0.018$ ) to 24 h (+3.35 nm); emission below 640 nm rises from 2 h ( $q = 0.018$ ). At 24 h the rise is smaller in  $cm/24-4$  ( $q = 0.036$ ).

# What the spectrum change looks like

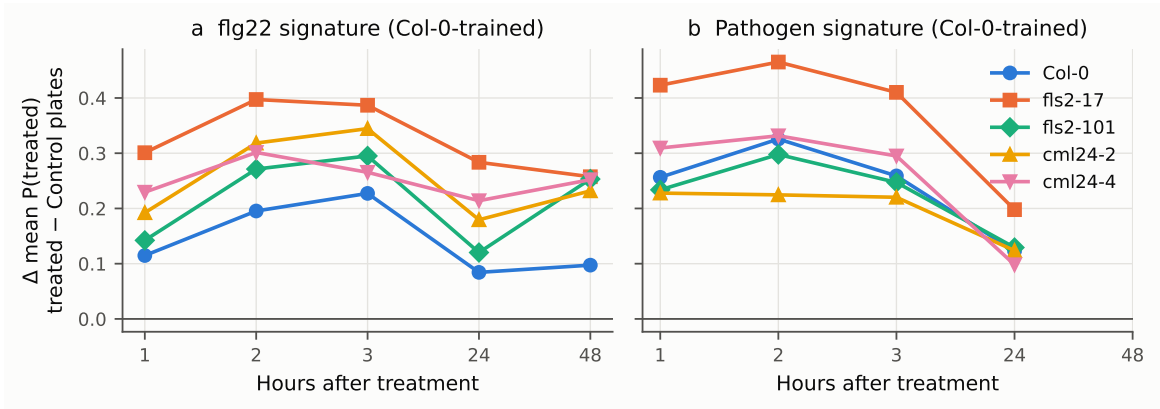


Area-normalised spectra, change from hour 0, treated minus Control (Col-0).

## Infected plates can be told apart from their spectra

- Leave-one-plate-out classifier, change-from-baseline spectra (Col-0)
- Plate-level AUC 1.00 at 1 h, 1.00 at 2 h, 1.00 at 24 h
- Exact permutation test over all 70 plate relabellings:  $p = 0.029$
- Weaker at 3 h (AUC 0.75,  $p = 0.143$ )

# The flg22 signal is not FLS2-dependent

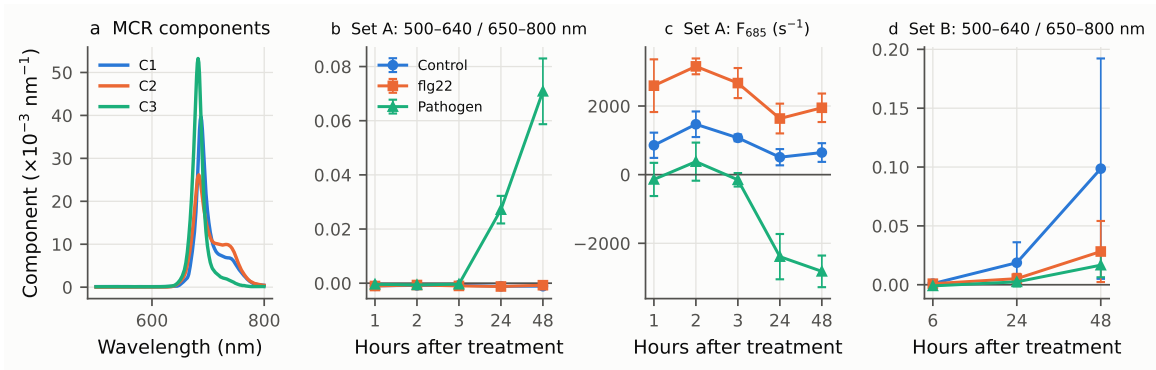


A Col-0-trained flg22 model fires as strongly in *fls2-17* (0.36) and *fls2-101* (0.24) as in Col-0 (0.18): a plate-level effect, not perception. The pathogen signature is in every genotype, as expected.

# The November replicate

- 405 nm excitation; Col-0, *fls2-17*, *fls2-101*; 0, 6, 24, 48 h
- Hand-drawn ROIs exist for only one plate per treatment, so treatment effects are confounded with plate there
- Within plates: flg22 shifts Col-0 emission shape differently from *fls2-17* at 6 h ( $q = 0.026$ ), the FLS2-dependent pattern
- Raw cubes (2–3 plates/treatment): no replicated treatment effect; one deteriorating Control plate had the largest short-wavelength rise
- Bacteria-only scans do not fluoresce: the signal is the plant's

# Every seedling in the raw cubes



1,623 seedlings, plate means, change from hour 0. The early signature replicates; 685 nm emission falls from 3 h ( $-3,462 \text{ s}^{-1}$  by 48 h) and seedlings shrink. Curve resolution finds no separate broad autofluorescence component.

# Take-home

- ❶ Carry acquisition metadata with every intensity: one camera setting made the most striking 2016 pattern.
- ❷ The plate is the experimental unit: image a baseline and validate leave-plate-out.
- ❸ Use non-perceiving mutants as the decisive control.
- ❹ Check every export: an un-subtracted dark level mimicked autofluorescence.
- ❺ A pathogen signature appears from 1 h (broader red peak), then chlorophyll emission falls and tissue is lost; short-wavelength emission marks damage, not infection.
- ❻ No robust CML24 effect.

# FAIR resource

- Code, manifests, hand-ROI spectra, derived tables:  
`github.com/dr-richard-barker/PathoFluor-At`
- Raw cubes (lossless, SHA-256 verified): Zenodo **[PENDING: DOIs]**
- Project page: `dr-richard-barker.github.io/PathoFluor-At`
- Every number in the paper and these slides is generated from the committed tables