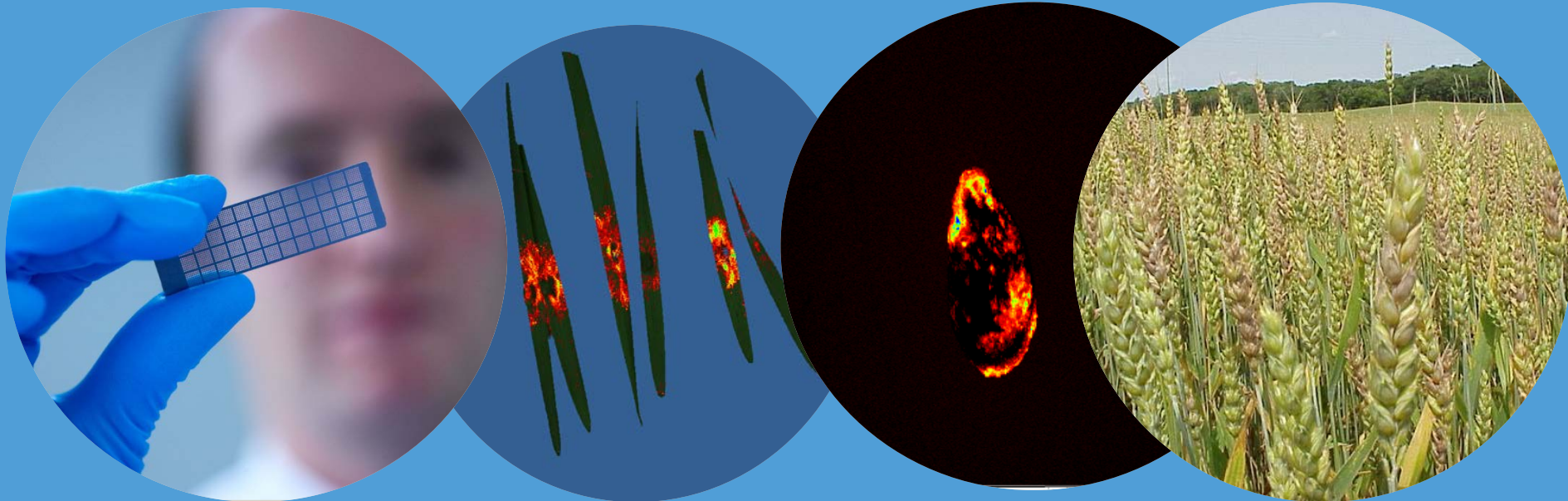


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# PathoScreen, a new approach in non-destructive quantitative detection

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Els Verstappen, Peter Bonants, Carolien Zijlstra and Theo van der Lee  
TESTA meeting 1 December 2015, Angers, France



# Drawbacks of conventional phenotyping

- Subjective

dress blue and black or white and gold

Ongeveer 131.000.000 resultaten (0,24 seconden)

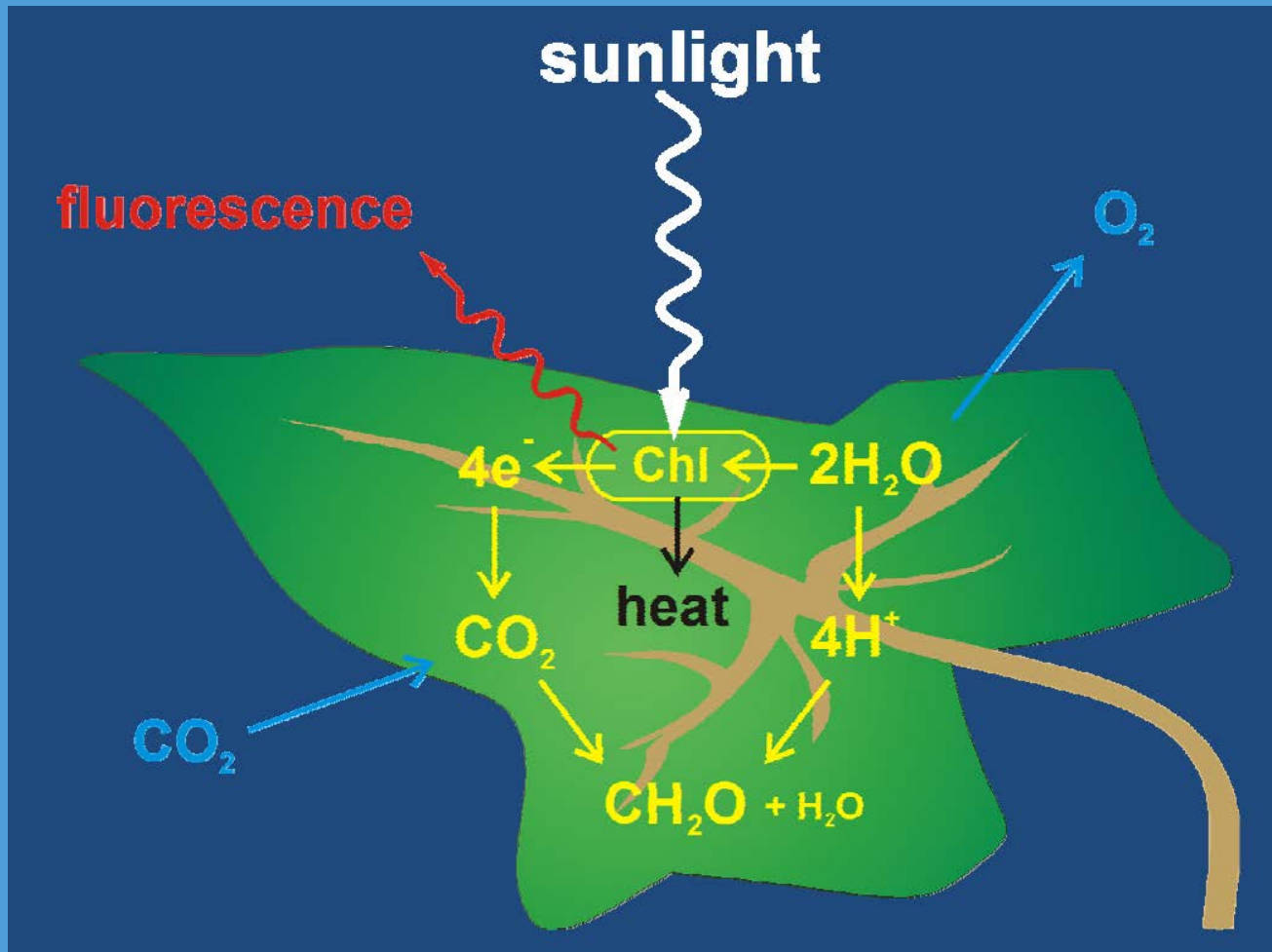


# Drawbacks of conventional Phenotyping

- Subjective
- Time consuming
- Extreme data reduction (scale 1-5)
- Visual symptoms do not tell the whole story
  - Pre-symptomatic phase (early measurement)
  - Resistance based on tolerance/hypersensitive response
  - Variability of the symptoms based on environmental conditions
  - Biomass of the pathogen is important

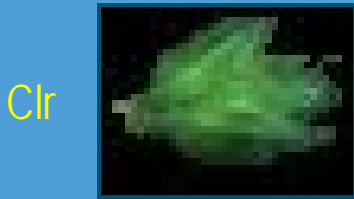
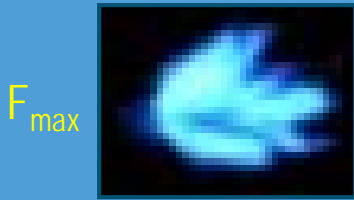
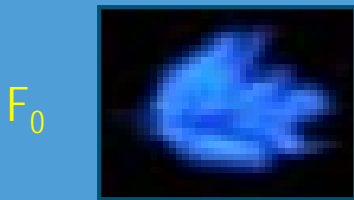


# Monitoring biotic stress by Chlorophyll fluorescence

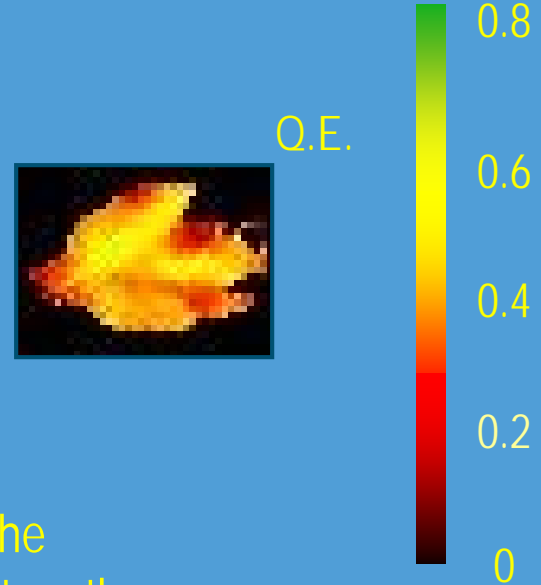


# Fusarium head blight on wheat (*Fusarium graminearum*)

Record three images



Calculate:  $F_v/F_m$  Efficiency of Photosynthesis



Colour table of  $F_v/F_m$

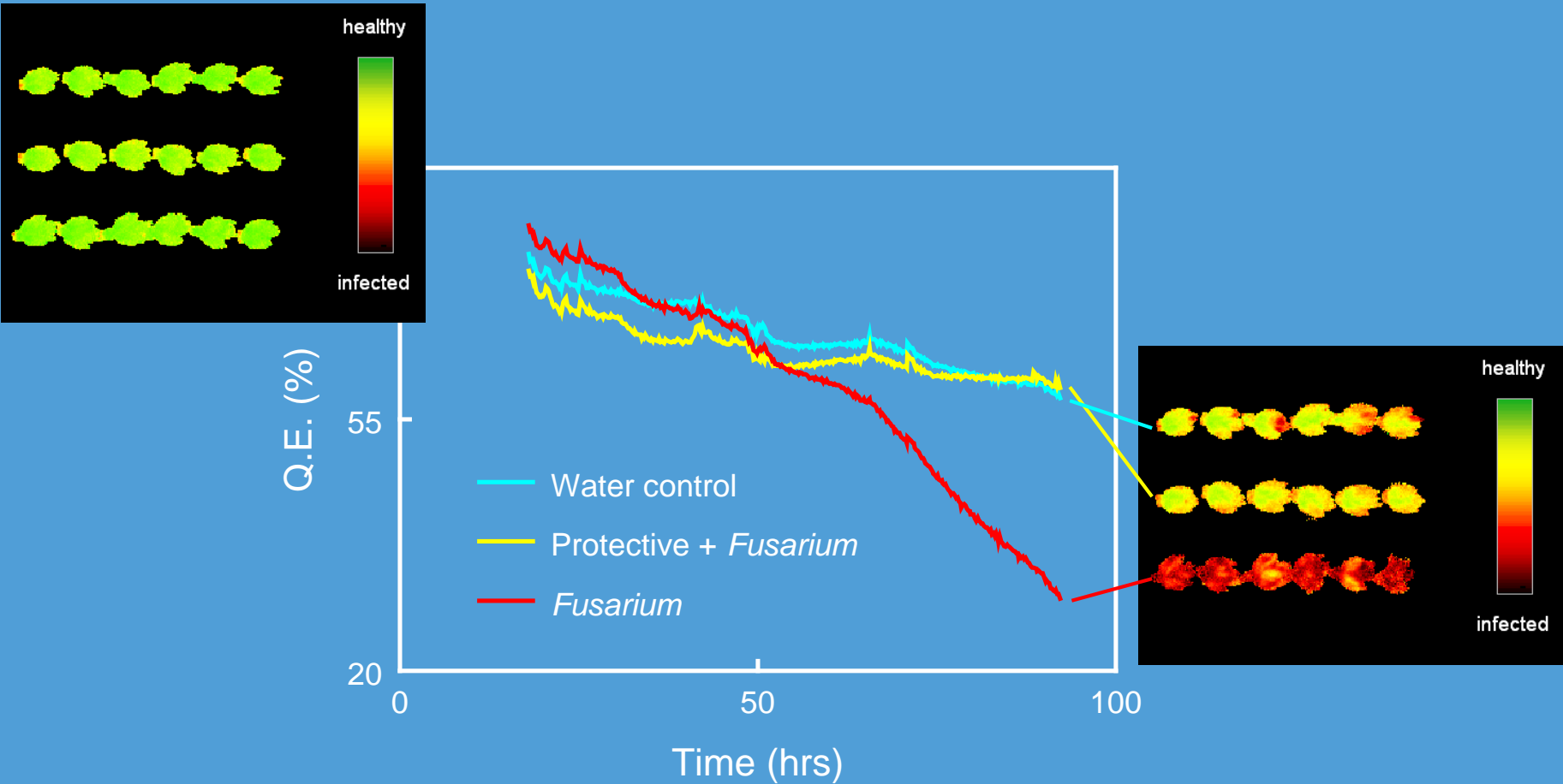
Image of  $F_v/F_m$  depends on the activity of photosynthesis - not on the amount of chlorophyll

*Lower  $F_v/F_m$  means more stress*





# Change in plant stress (Fv/Fm) in time



# Septoria Leaf Blotch

6.000.000 x 65.536 x 7 x time



pre-symptomatic phase (early measurement)

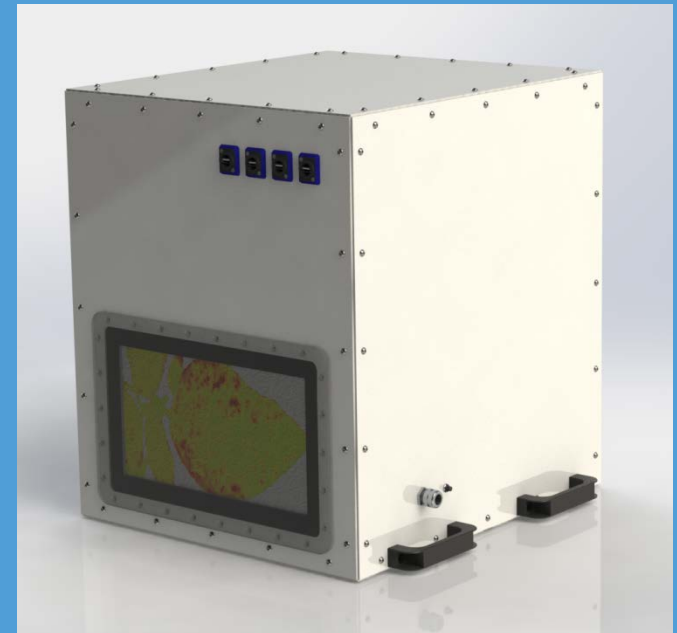
biotrophic, necrotrophic phase



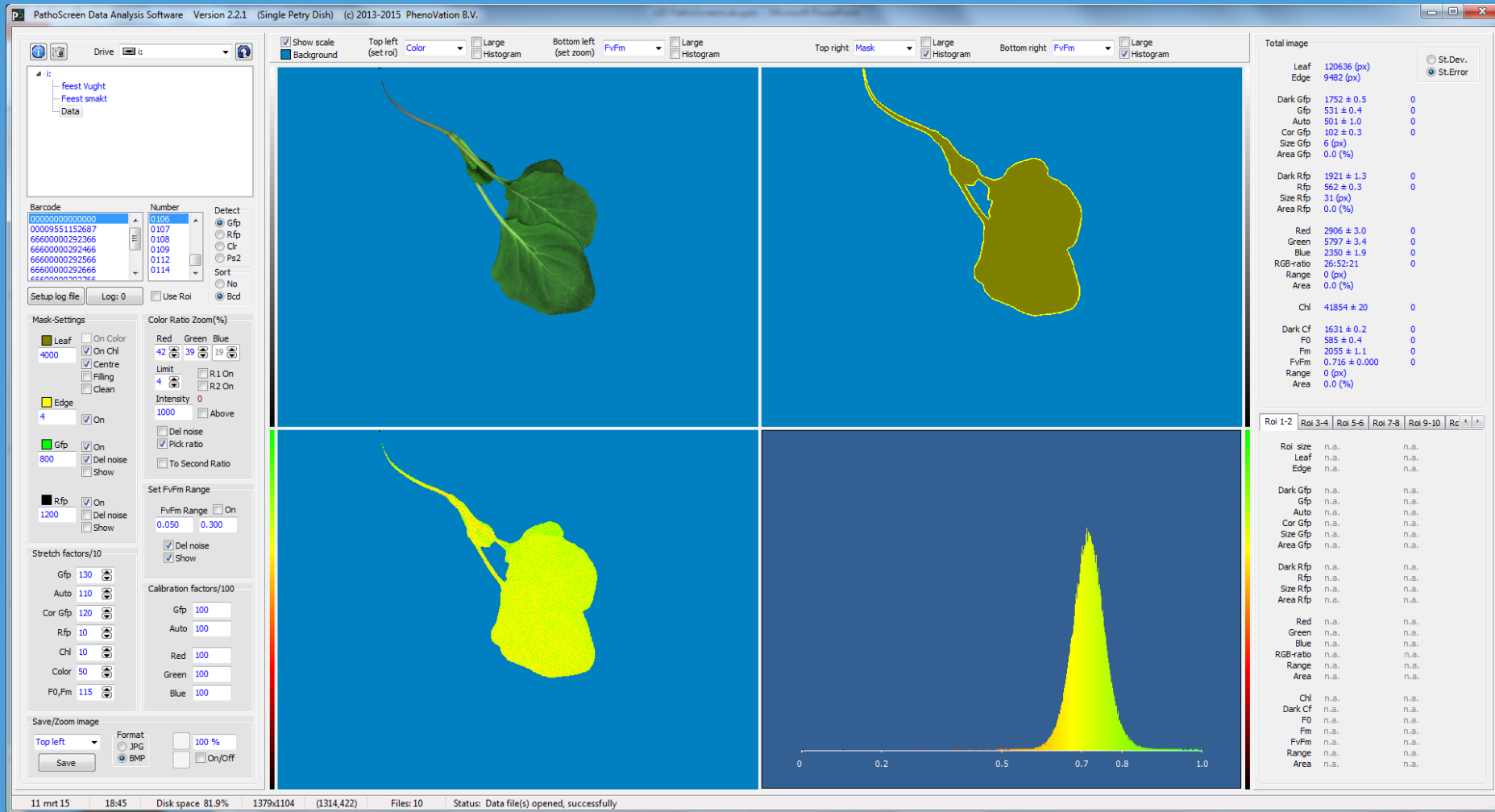


# The instrumentation

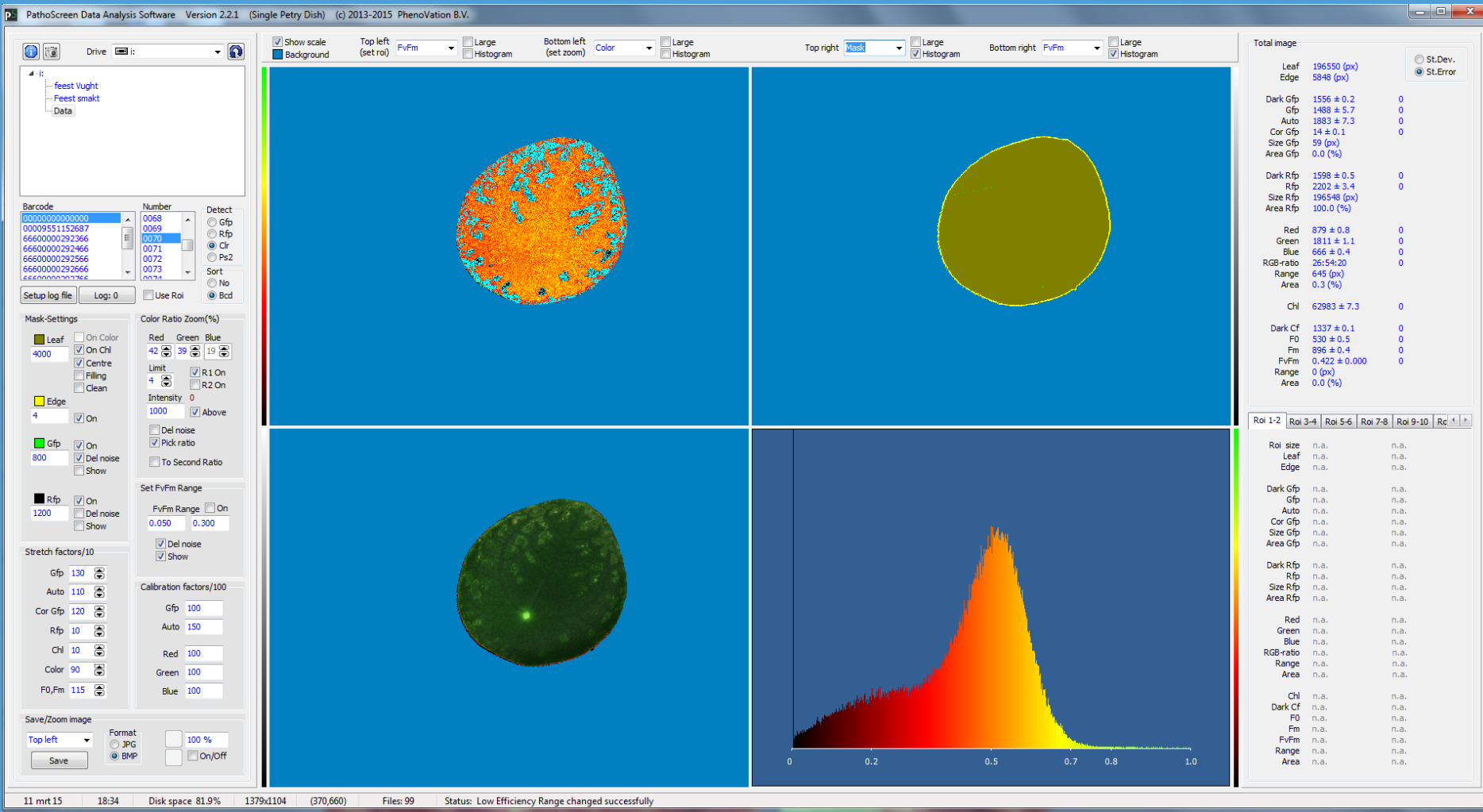
- **Automated imaging system** for the determination of the biomass of GFP-transformed pathogens in combination with the measurement of photosynthetic activity and colour imaging (necrosis, chlorosis).
- **High resolution imaging** (130x130 mm), petry dishes, plant leafs, plantlets can be imaged in a single frame.
- **Fast** (5-10 seconds for 7 measurements including filter changes)
- The LED-PathoScreenLab is equipped with a **barcode reader** and data files are stored with the corresponding barcode.
- **Colour images** (red, green and blue).
- **Wireless** mouse and keyboard, passive cooling
- **Personal** storage space and settings data analysis on your own computer
- Possibility to generate **movies**
- **Actinic** light up to 100  $\mu\text{mol}$
- **High intensity**; up to 4 times the sun
- Can be **transported** to your samples



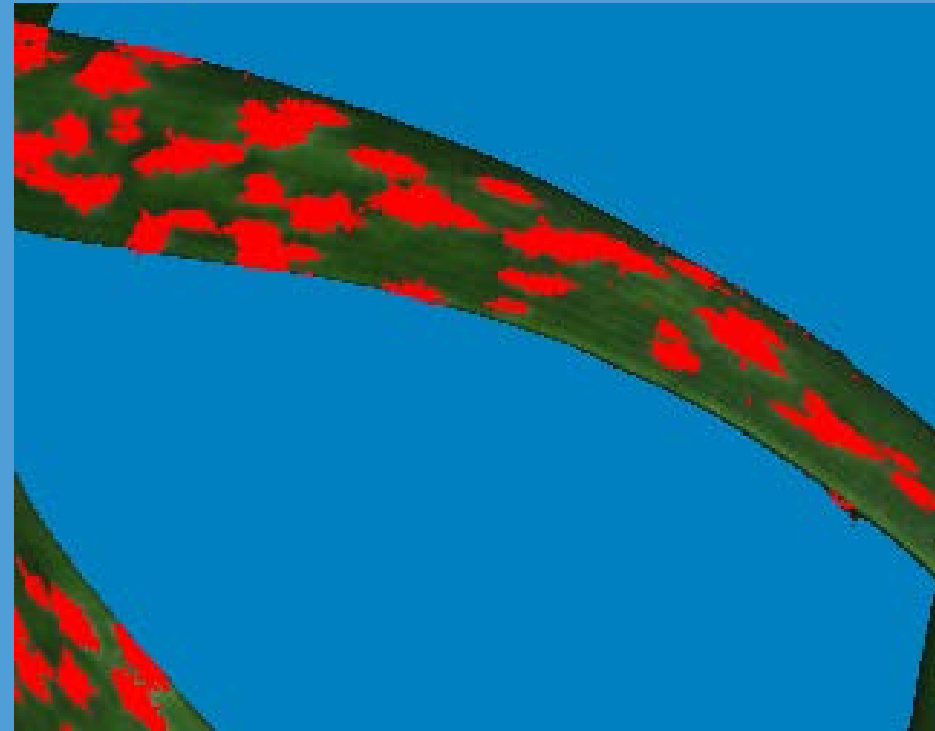
# A healthy cabbage plant



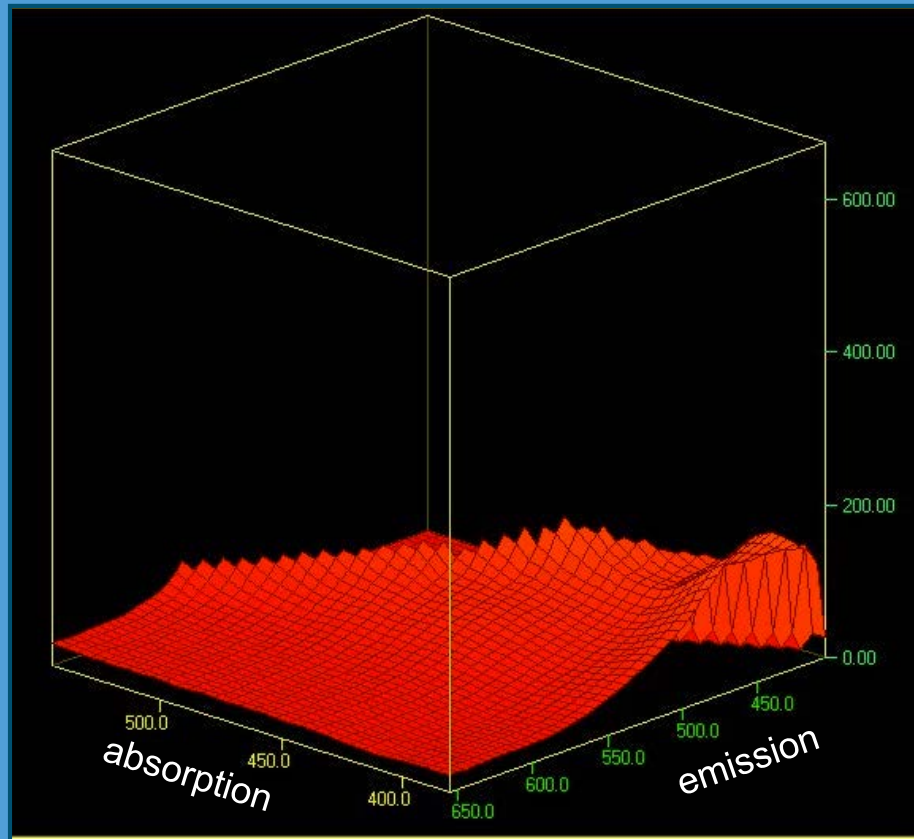
# Plant growing in sub-optimal conditions (Tempora)



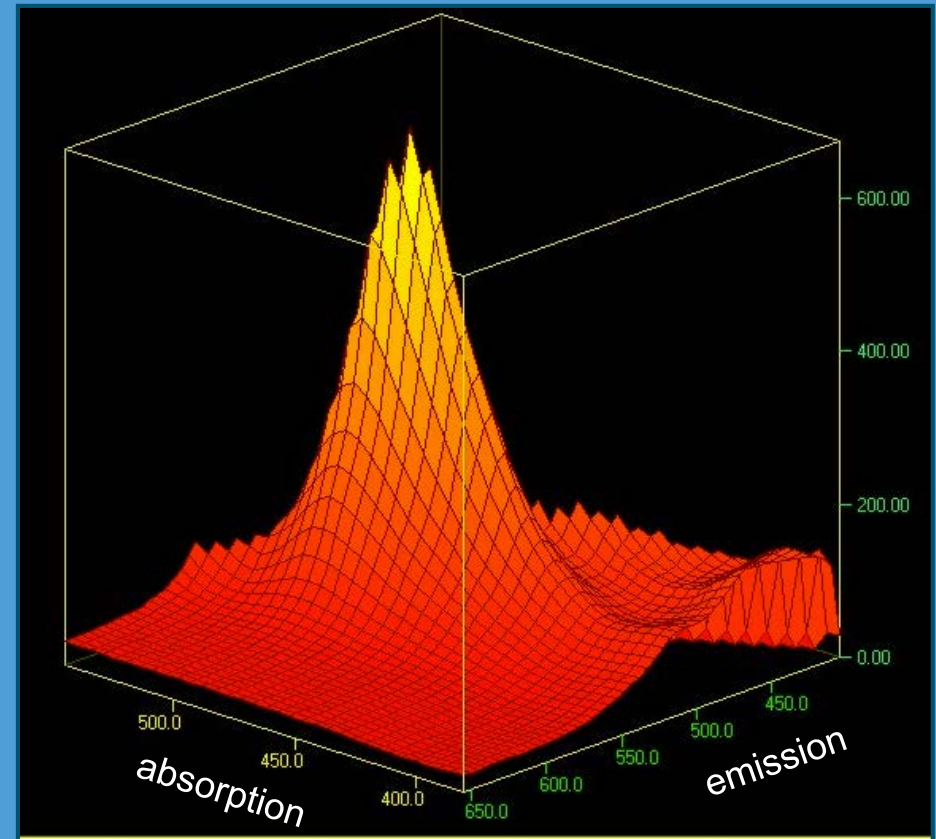
# Digital imaging in the visual spectrum



# PathoScreen tracking of GFP tagged isolates



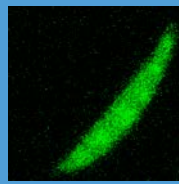
wt



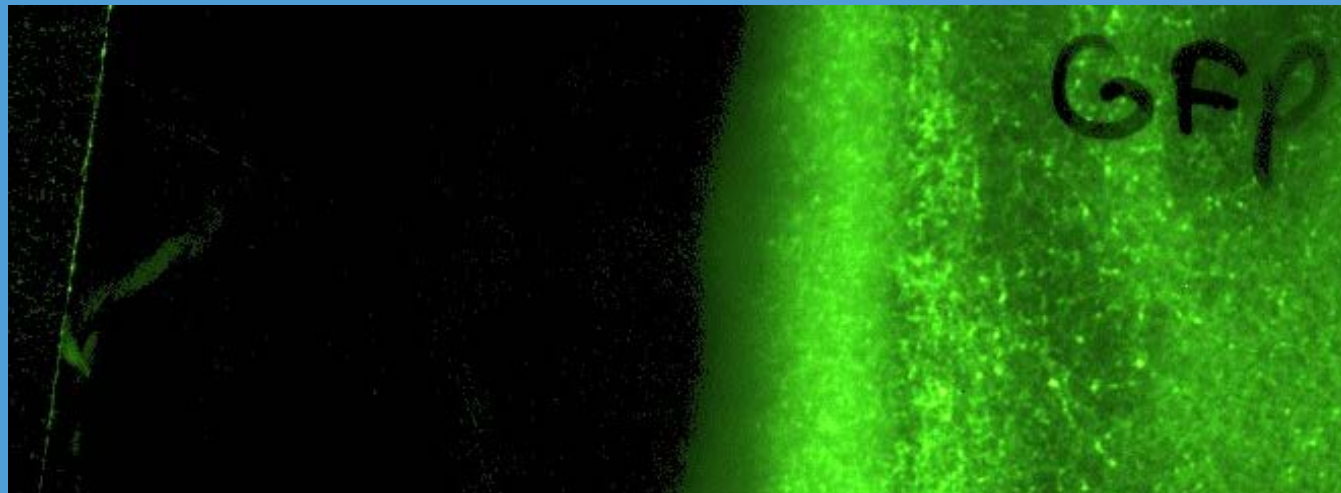
GFP



# PathoScreen



Day-light

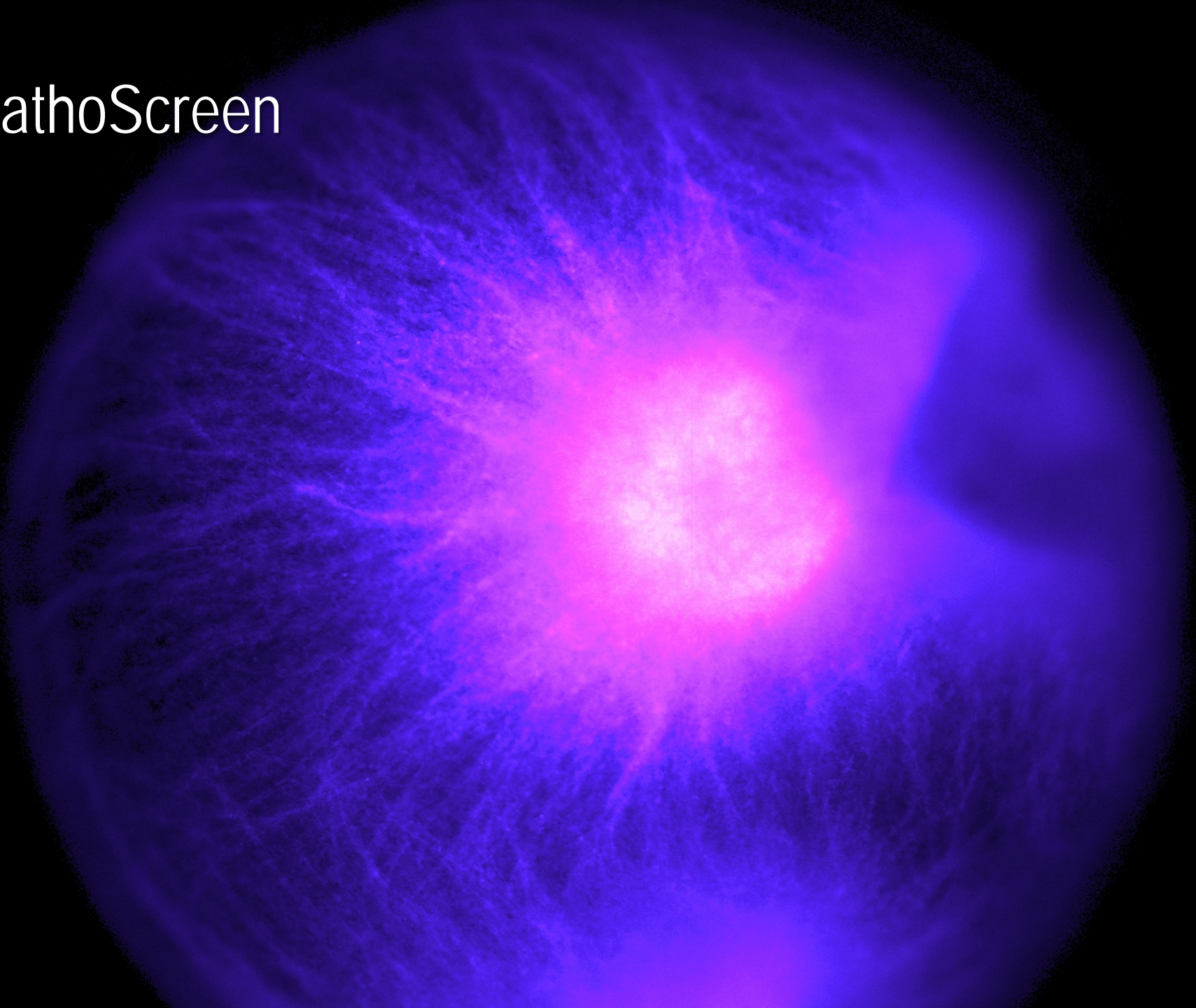


Green screen



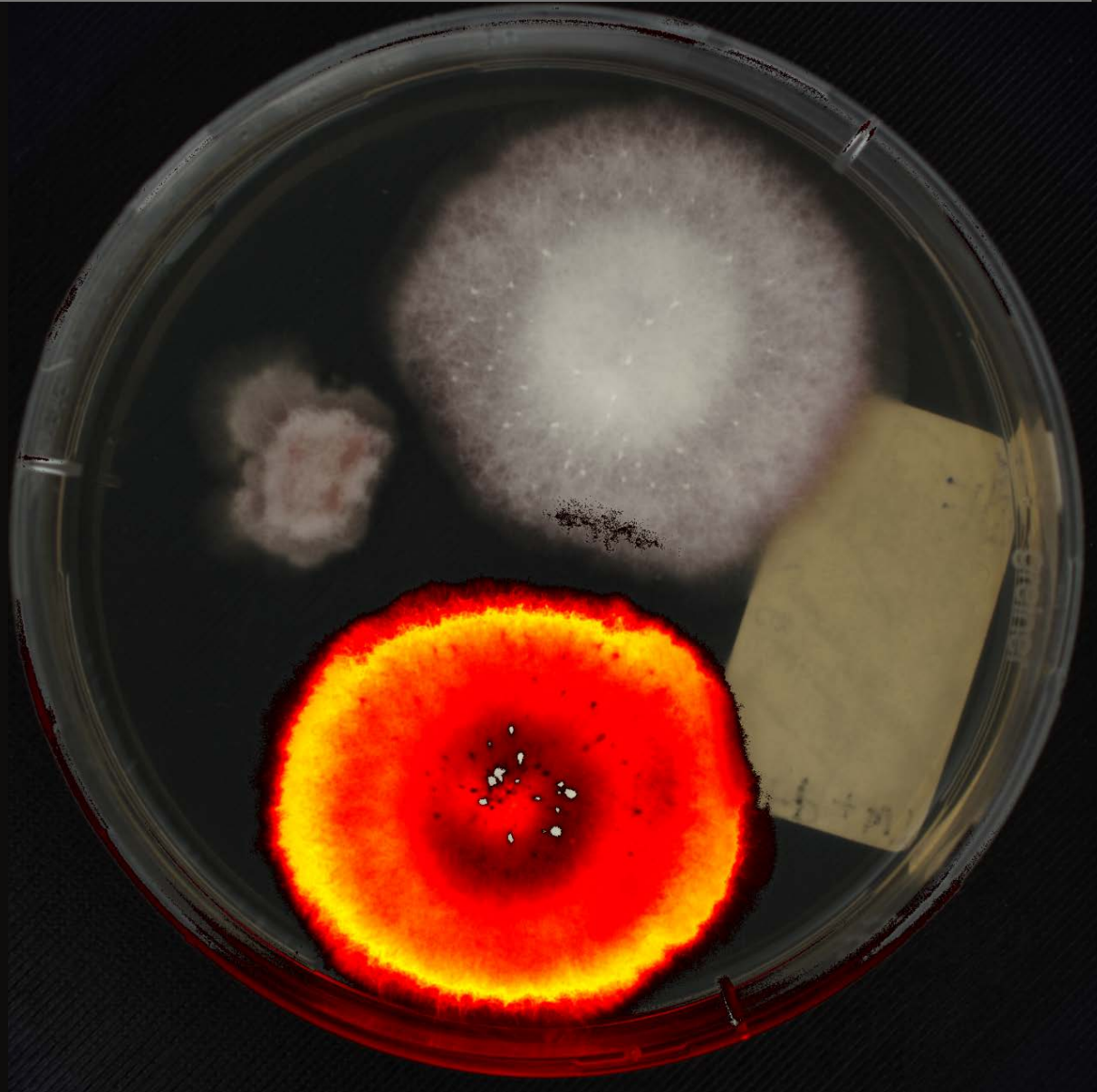


PathoScreen



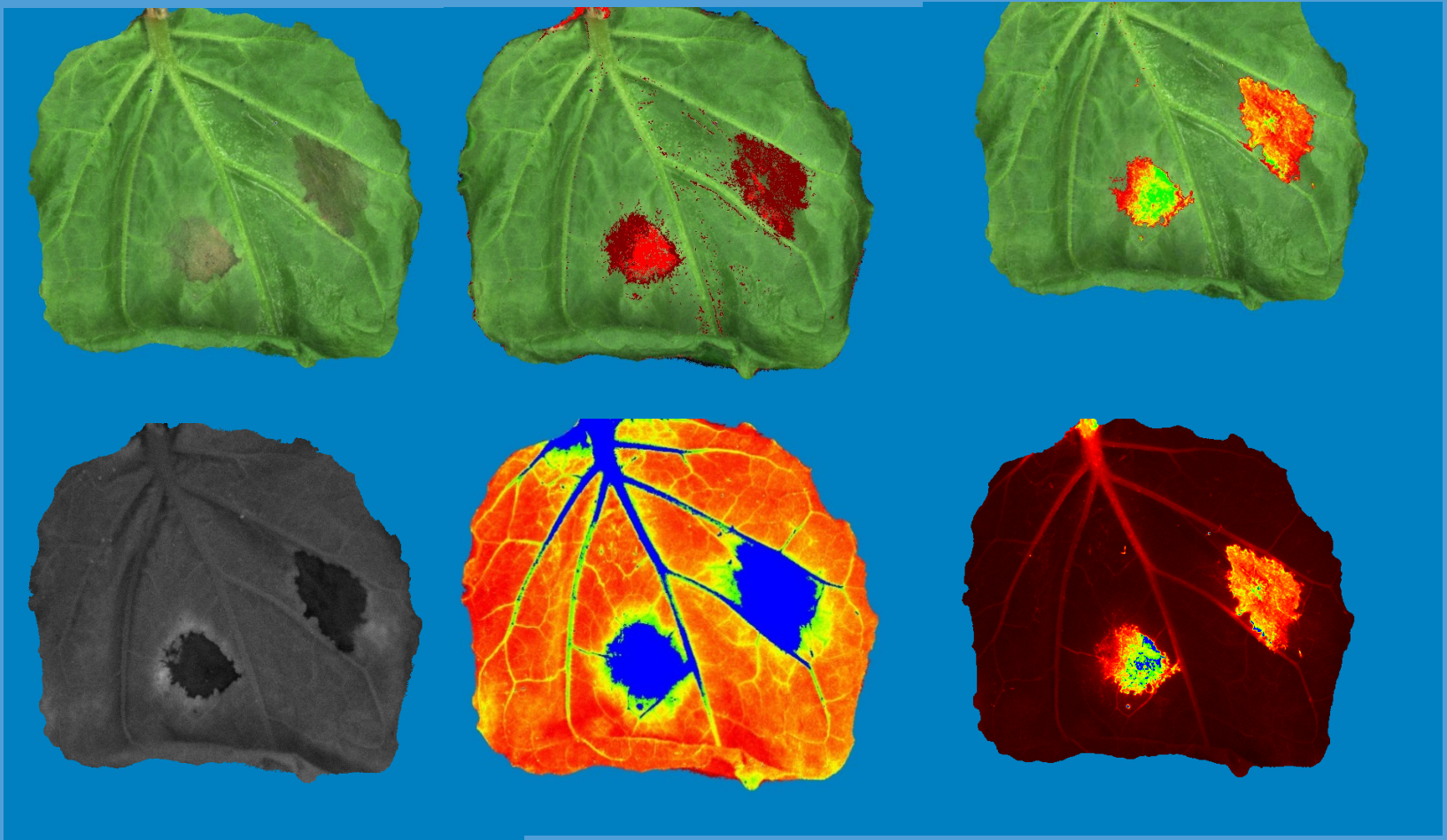
# GFP or RFP tagged pathogens

- Clean overlay
- No pixel shift





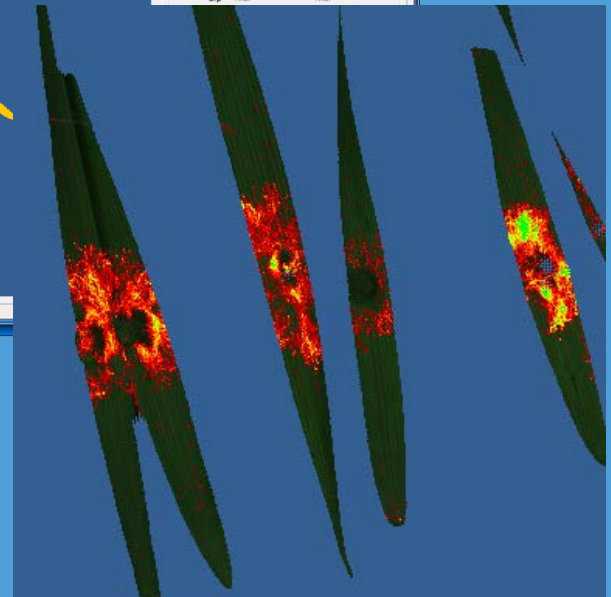
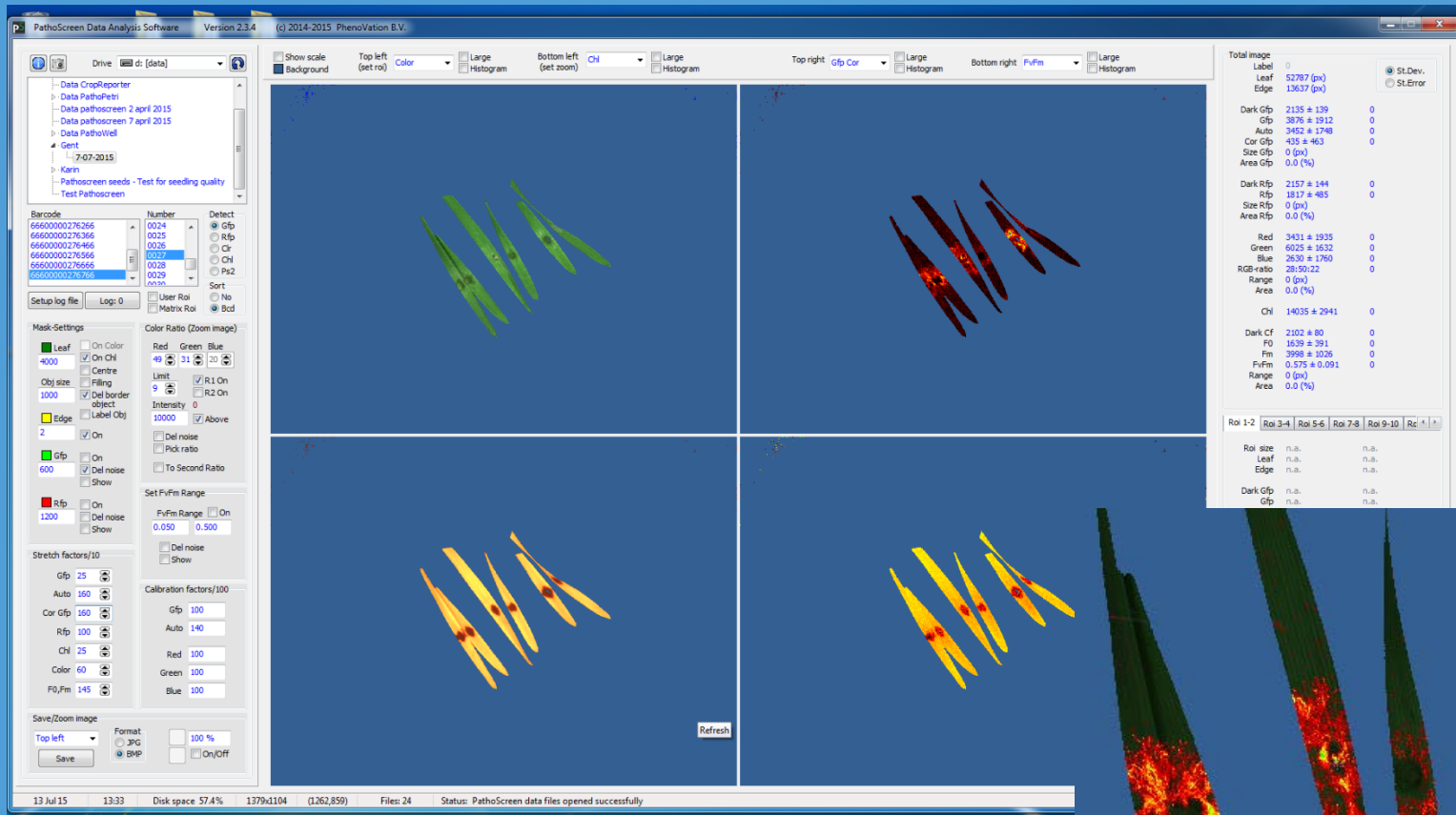
# Multi dimensional imaging



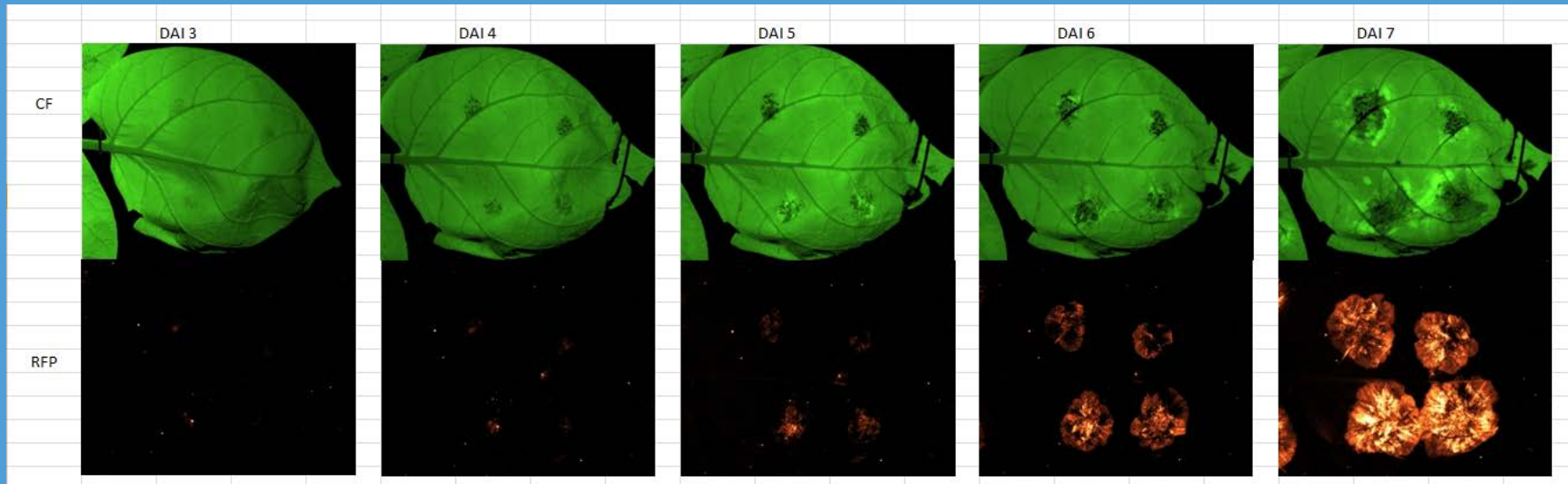
*P. infestans* infection on *N. benthamiana*



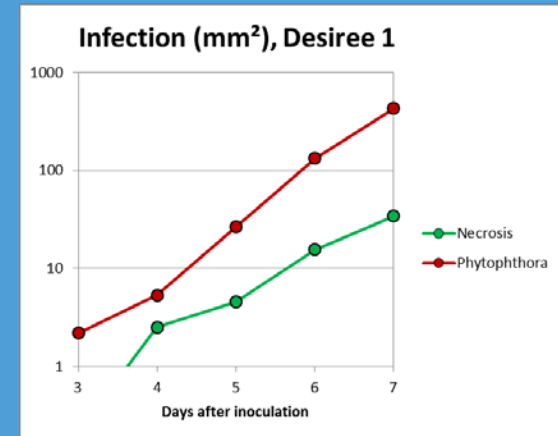
# Fusarium infection on wheat leaves



# Multidimensional screening for Potato late blight



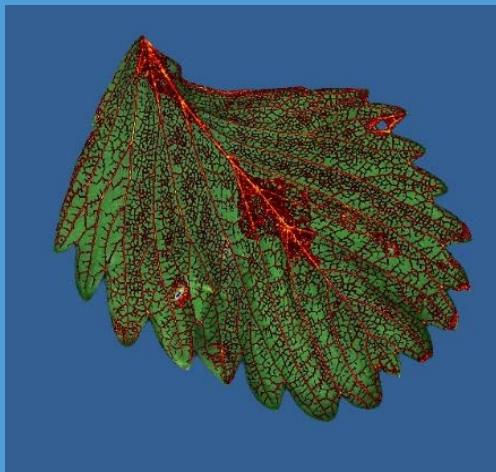
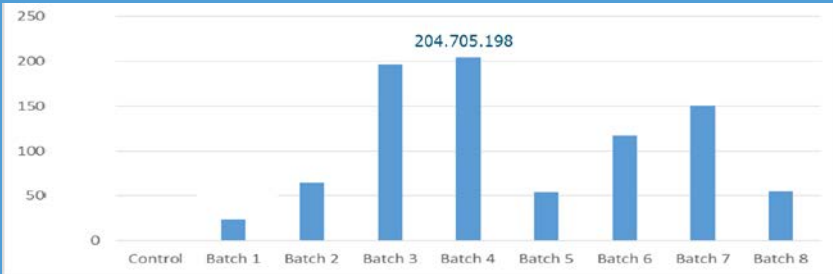
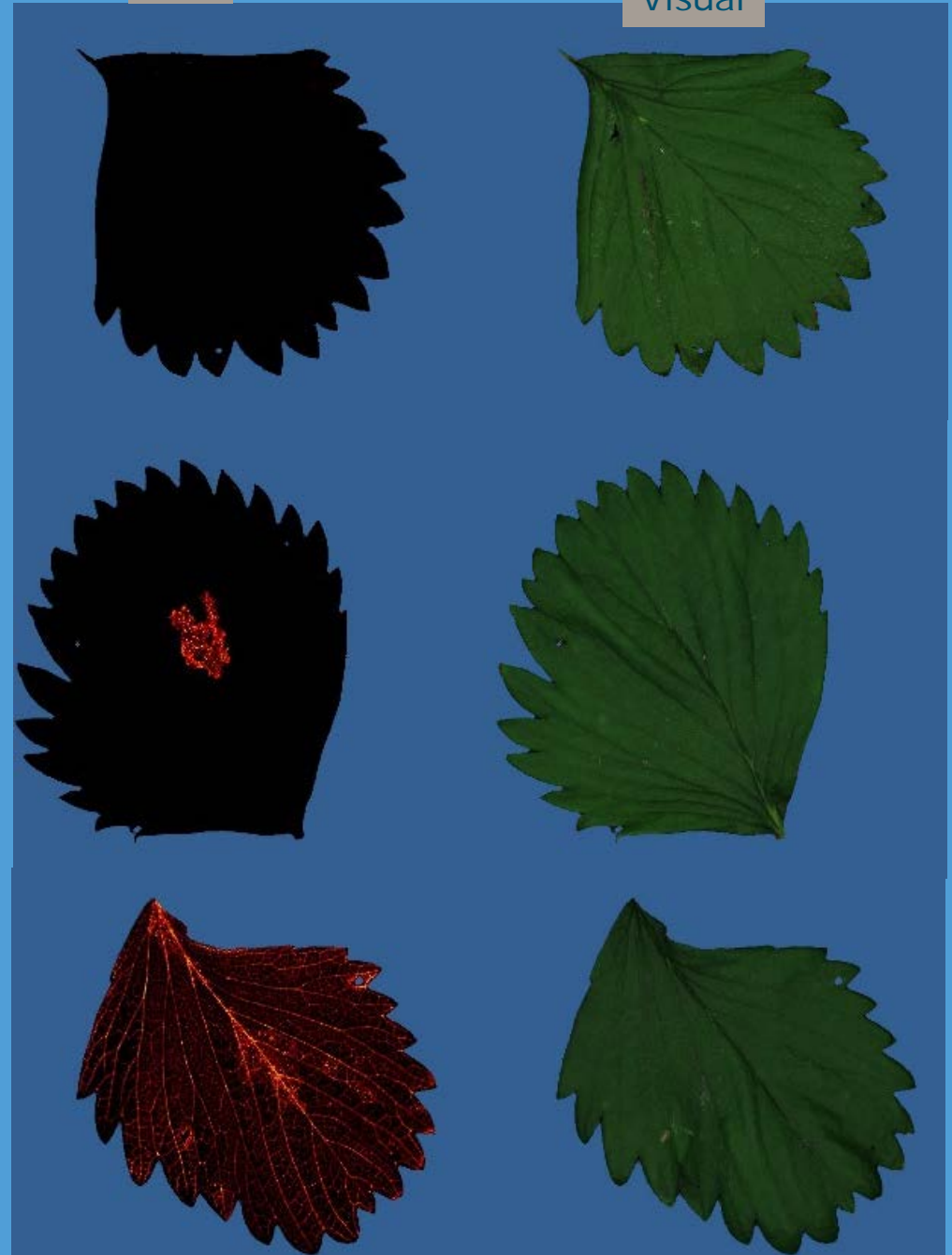
- *Phytophthora infestans*
- Causal agent of Potato/tomato late blight
- Major target agrochemical industry
- Searching for resistance
- QTL-analysis



# Fungicide application

BSF

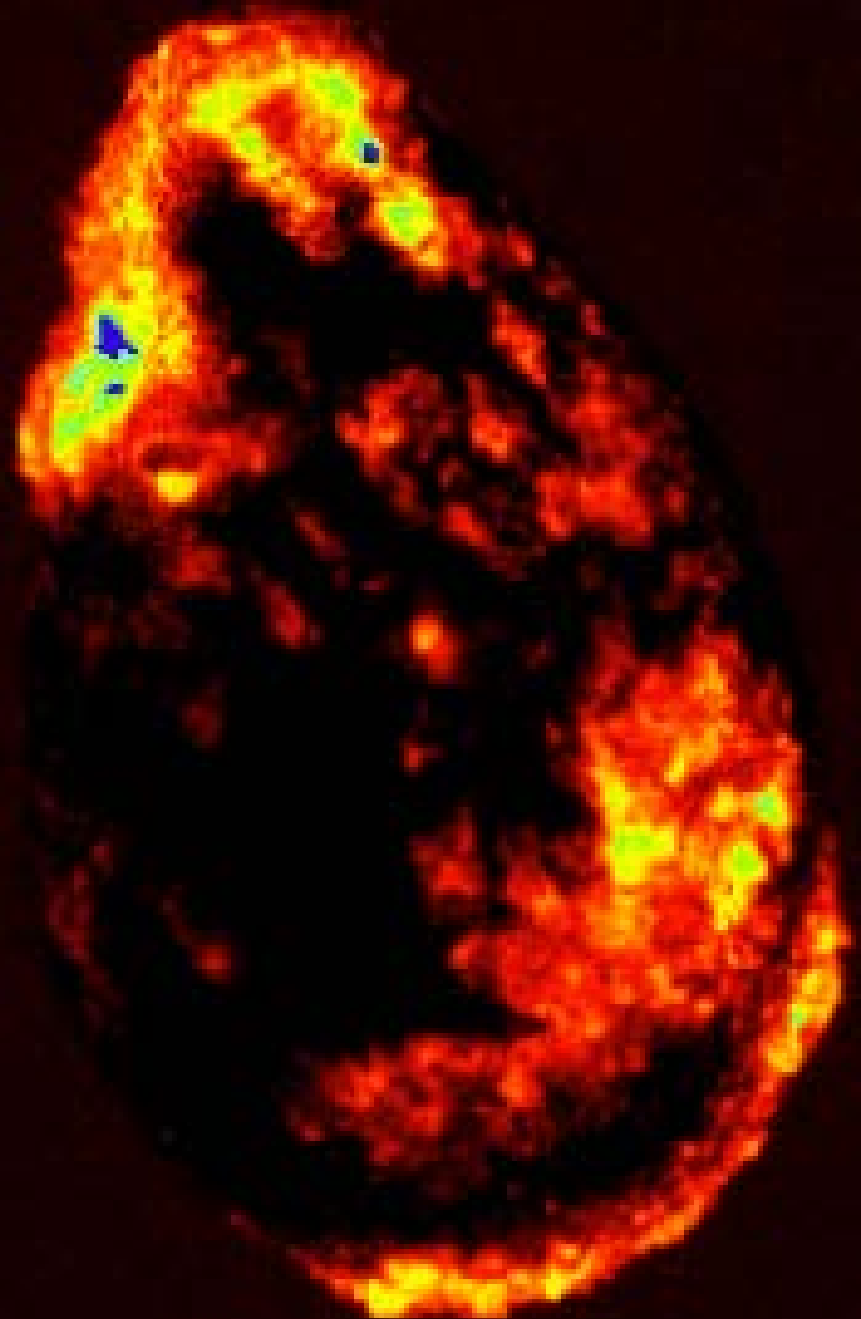
Visual





# Summary

- Parameters:
  - Growth: projected leaf area in pixels.
  - Quantification of biotic or abiotic stress
  - Quantification of chlorosis and/or necrosis
  - Mean Intensity with standard deviation
- Output:
  - Data output in CSV-format
  - Images with overlay



# Acknowledgements

Vivianne Vleeshouwers

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Olga Scholten

Gert Kema

Cees Waalwijk





abstract:

For many studies the correct assessment of the presence of a particular pathogen as well as the stage/spread of the infection in plants is critical. Currently, assessment of infection of pathogens is complicated and scoring often relies on manual recording of visual symptoms. However, this scoring is subjective, time consuming, results in extreme data reduction whereby the original data are lost making reanalysis of the data impossible. In addition plant pathogen interactions often involve a latent infection phase for which no visual symptoms can be recorded. Consequently, there is a strong need for development of new procedures. In the last decades we have been experimenting with different procedures and identified that correct colour interpretation (chlorosis, necrosis), measurement of plant stress by chlorophyll fluorescence (fv/fm), quantifying the amount of chlorophyll and direct visualization of pathogens labelled with GFP or RFP can be extremely useful to monitor the various stages in the infection process. In addition, as plant pathogens often show localized infection with limited pathogen biomass, a high resolution combined with a high sensitivity is required. Based on these requirements we have initiated the development of the PathoScreen platform that allows fast multi-dimensional Digital Phenotyping of plant pathogen interactions. Examples of it's use will be shown.

