Spectral Imaging for Plant Phenotyping

NMBU NOVA PhD course

15 • June • 2023, Gerrit Polder

Photosyntech

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Introduction

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Introduction

- Gerrit Polder,
  - 30 years at Wageningen University & Research.
  - Senior scientist computer vision for plant phenotyping
- Background: Electronics/Applied Physics.
  - PhD on Spectral Imaging
- Aim of this lecture:
  - To introduce spectral imaging and show its value to applications in plant phenotyping.

Contact: gerrit.polder@wur.nl

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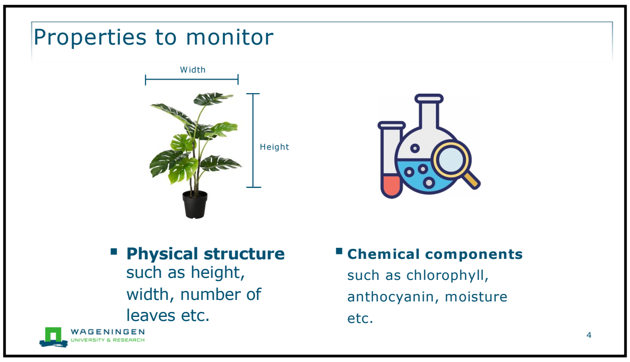
Properties to monitor
Width
Height
Physical structure such as height, width, number of leaves etc.
Chemical components such as chlorophyll, anthocyanin, moisture etc.
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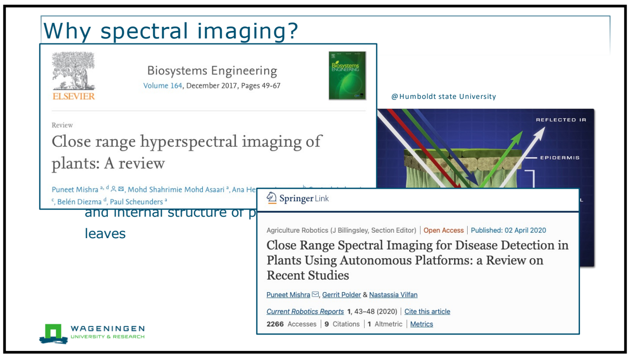
Why spectral imaging?
ELSEVIER
Biosystems Engineering
Volume 164, December 2017, Pages 49-67
Review
Close range hyperspectral imaging of plants: A review
and internal structure of p
leaves
@Humboldt state University
REFLECTED IR
EPIDERMIS
SpringerLink
Agriculture Robotics (J Billingsley, Section Editor) | Open Access | Published: 02 April 2020
Close Range Spectral Imaging for Disease Detection in Plants Using Autonomous Platforms: a Review on Recent Studies
Puneet Mishra, Gerrit Polder & Nastassia Vilfan
Current Robotics Reports 1, 43–48 (2020) | Cite this article
2266 Accesses | 9 Citations | 1 Altmetric | Metrics
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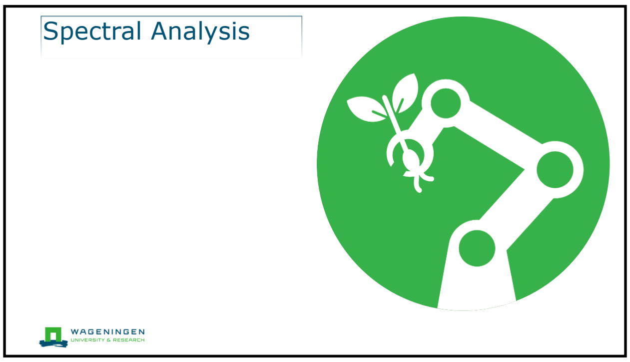
Spectral Analysis
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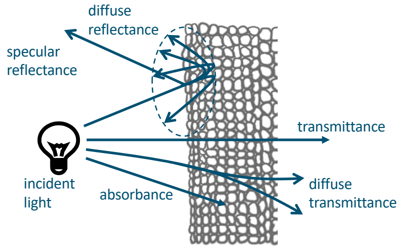
Interaction of light with matter



7

Light and the Electromagnetic Spectrum

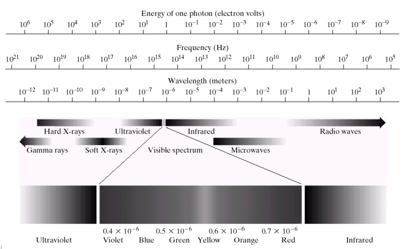


FIGURE 2.10 The electromagnetic spectrum. The visible spectrum is shown zoomed to facilitate explanation, but note that the visible spectrum is a rather narrow portion of the EM spectrum.

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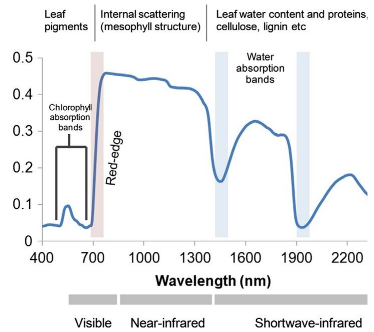
Spectral features



9


Spectral Imaging
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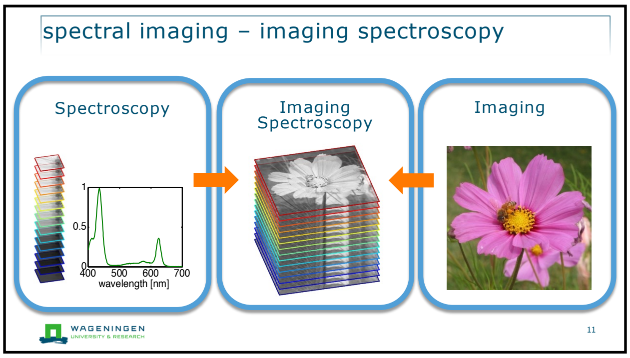
spectral imaging – imaging spectroscopy
Spectroscopy
Imaging Spectroscopy
Imaging
1
0.5
0
400 500 600 700
wavelength [nm]
WAGENINGEN
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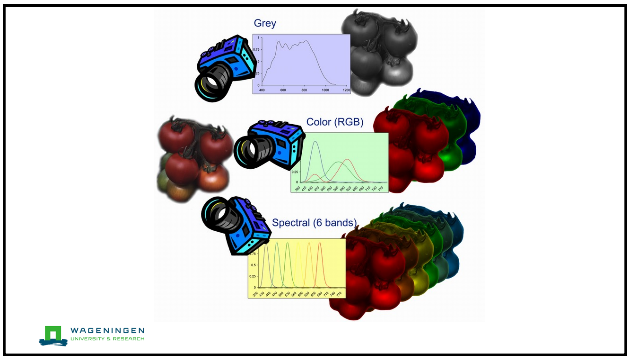
Grey
Color (RGB)
Spectral (6 bands)
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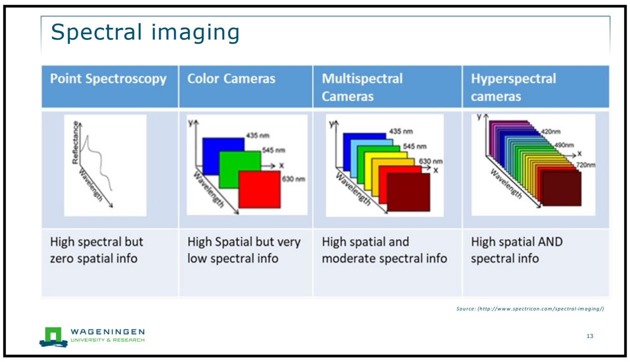
Spectral imaging
Point Spectroscopy
Color Cameras
Multispectral Cameras
Hyperspectral cameras
Reflectance
Wavelength
435 nm
545 nm
630 nm
420nm
490nm
720nm
High spectral but zero spatial info
High Spatial but very low spectral info
High spatial and moderate spectral info
High spatial AND spectral info
Source: (http://www.spectricon.com/spectral-imaging/)
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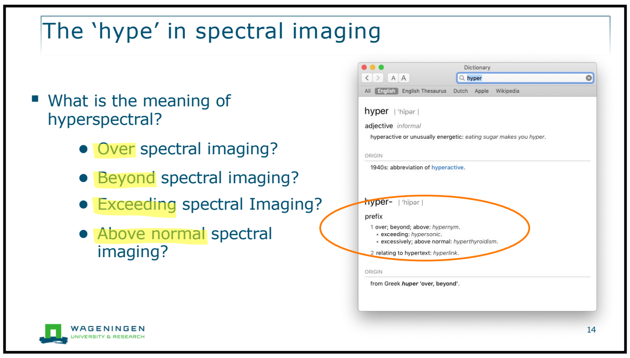
The ‘hype’ in spectral imaging
What is the meaning of hyperspectral?
Over spectral imaging?
Beyond spectral imaging?
Exceeding spectral Imaging?
Above normal spectral imaging?
Dictionary
hyper
All English English Thesaurus Dutch Apple Wikipedia
hyper | ˈhīpər |
adjective informal
hyperactive or unusually energetic: eating sugar makes you hyper.
ORIGIN
1940s: abbreviation of hyperactive.
hyper- | ˈhīpər |
prefix
1 over; beyond; above: hypernym.
• exceeding: hypersonic.
• excessively; above normal: hyperthyroidism.
2 relating to hypertext: hyperlink.
ORIGIN
from Greek huper ‘over, beyond’.
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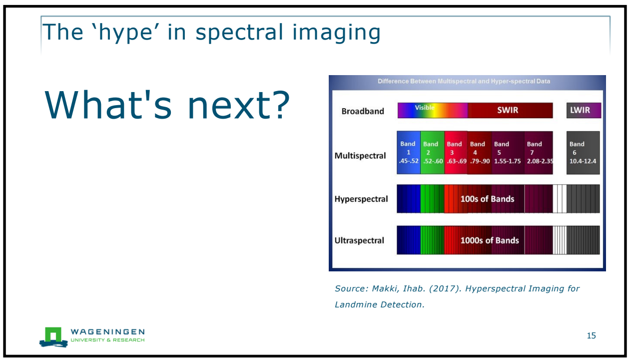
The ‘hype’ in spectral imaging
What's next?
Difference Between Multispectral and Hyper-spectral Data
Broadband
Visible
SWIR
LWIR
Multispectral
Band 1 .45-.52
Band 2 .52-.60
Band 3 .63-.69
Band 4 .79-.90
Band 5 1.55-1.75
Band 7 2.08-2.35
Band 6 10.4-12.4
Hyperspectral
100s of Bands
Ultraspectral
1000s of Bands
Source: Makki, Ihab. (2017). Hyperspectral Imaging for Landmine Detection.
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Orig
The t
imagi
1980'
comm
devel
Imagi
NASA
7 June 1985, Volume 228, Number 4704
SCIENCE
Imaging Spectrometry for Earth Remote Sensing
Alexander F. H. Goetz, Gregg Vane
Jerry E. Solomon, Barrett N. Rock
Remote sensing of the earth's surface from aircraft and from spacecraft provides information not easily acquired by surface observations. Until recently, the main limitations of remote sensing were that no subsurface information could be acquired and that surface information lacked specificity (1). Orbital imaging
portions of the spectrum. In addition, we discuss several approaches to the analysis of the resulting hyperspectral image data sets. Hyperspectral refers to the multidimensional character of the spectral data set. In the past, data were acquired in four to seven spectral bands. Imaging spectrometry now makes possi-
age-based
development
mage
4: 14.
on, J, et al.
try for Earth
228: 4704.
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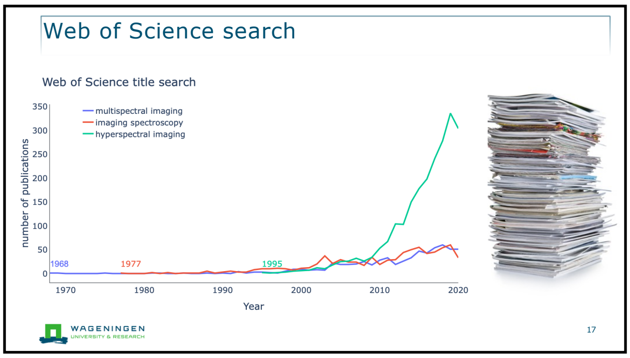
Web of Science search
Web of Science title search
multispectral imaging
imaging spectroscopy
hyperspectral imaging
number of publications
1968
1977
1995
Year
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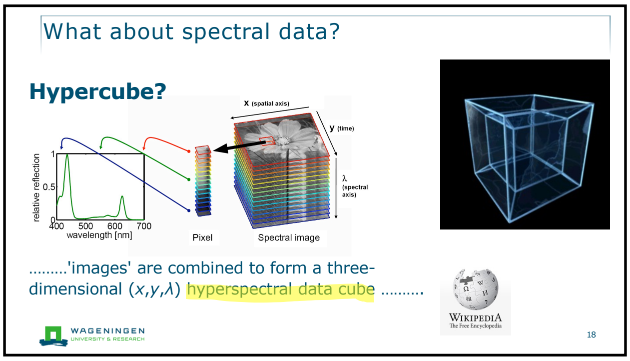
What about spectral data?
Hypercube?
x (spatial axis)
y (time)
λ (spectral axis)
relative reflection
wavelength [nm]
Pixel
Spectral image
.........'images' are combined to form a three-dimensional (x,y,λ) hyperspectral data cube ....
WIKIPEDIA The Free Encyclopedia
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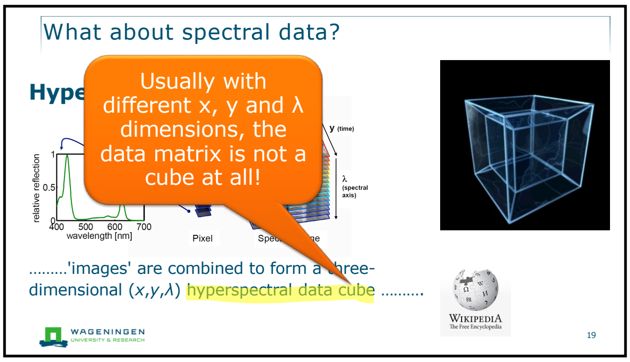
What about spectral data?
Hype
Usually with different x, y and λ dimensions, the data matrix is not a cube at all!
relative reflection
wavelength [nm]
Pixel
y (time)
λ (spectral axis)
.........'images' are combined to form a three-dimensional (x,y,λ) hyperspectral data cube ....
WIKIPEDIA
The Free Encyclopedia
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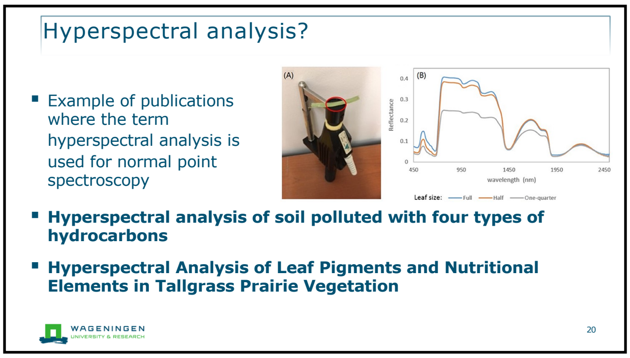
Hyperspectral analysis?
Example of publications where the term hyperspectral analysis is used for normal point spectroscopy
(A)
(B)
Reflectance
wavelength (nm)
Leaf size: Full Half One-quarter
Hyperspectral analysis of soil polluted with four types of hydrocarbons
Hyperspectral Analysis of Leaf Pigments and Nutritional Elements in Tallgrass Prairie Vegetation
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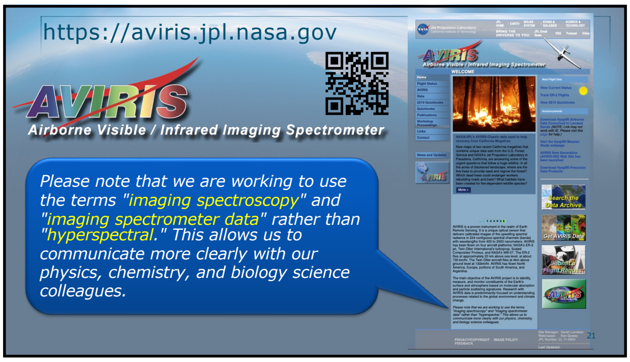
https://aviris.jpl.nasa.gov
AVIRIS
Airborne Visible / Infrared Imaging Spectrometer
Please note that we are working to use the terms "imaging spectroscopy" and "imaging spectrometer data" rather than "hyperspectral." This allows us to communicate more clearly with our physics, chemistry, and biology science colleagues.
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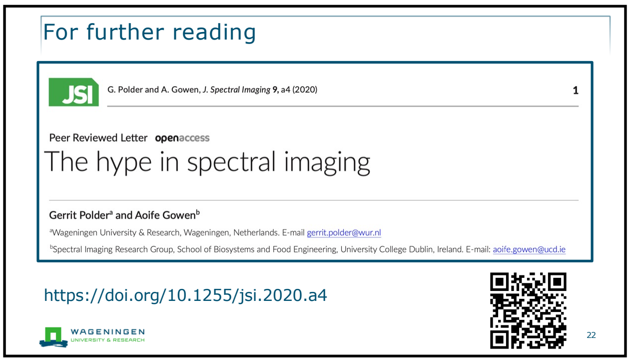
For further reading
JSI
G. Polder and A. Gowen, J. Spectral Imaging 9, a4 (2020)
1
Peer Reviewed Letter openaccess
The hype in spectral imaging
Gerrit Polder[a] and Aoife Gowen[b]
[a]Wageningen University & Research, Wageningen, Netherlands. E-mail gerrit.polder@wur.nl
[b]Spectral Imaging Research Group, School of Biosystems and Food Engineering, University College Dublin, Ireland. E-mail: aoife.gowen@ucd.ie
https://doi.org/10.1255/jsi.2020.a4
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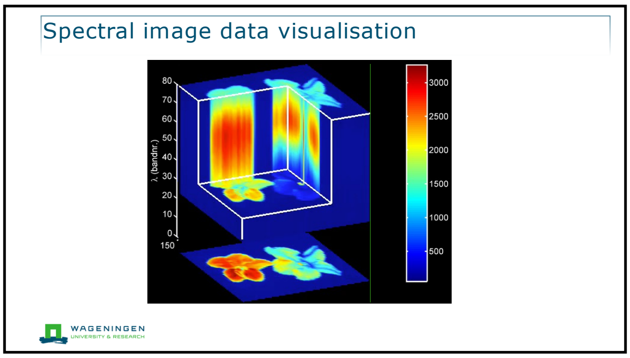
Spectral image data visualisation
80
70
60
50
40
30
20
10
0
150
λ (bandnr.)
3000
2500
2000
1500
1000
500
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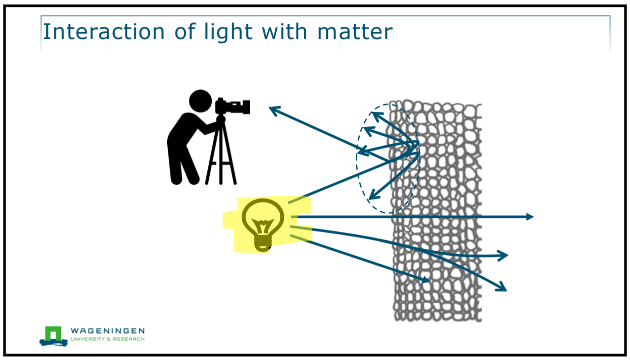
Interaction of light with matter
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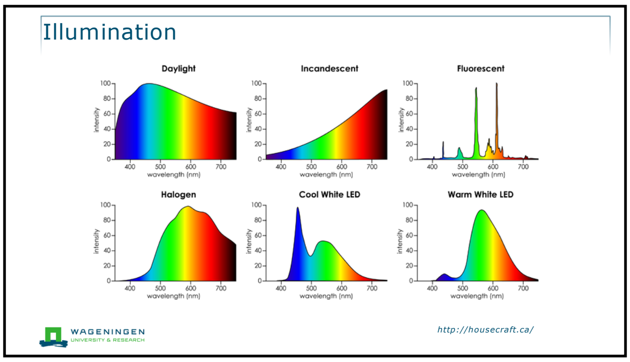
Illumination
Daylight
Incandescent
Fluorescent
Halogen
Cool White LED
Warm White LED
wavelength (nm)
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http://housecraft.ca/

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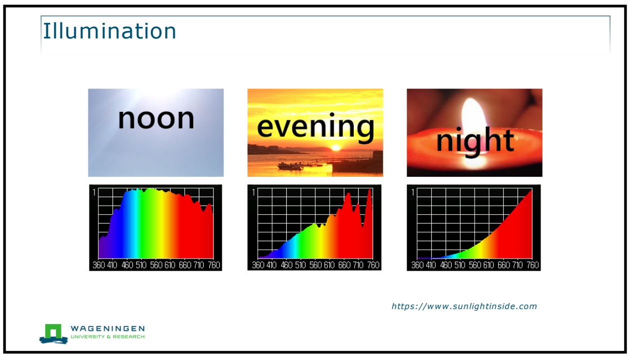
Illumination
noon
evening
night
360 410 460 510 560 610 660 710 760
https://www.sunlightinside.com
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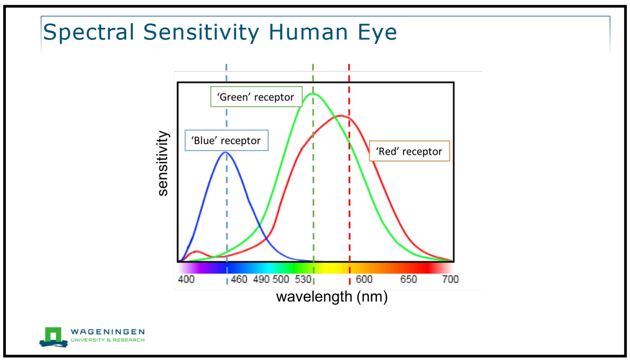
Spectral Sensitivity Human Eye
'Green' receptor
'Blue' receptor
'Red' receptor
sensitivity
400 460 490 500 530 600 650 700
wavelength (nm)
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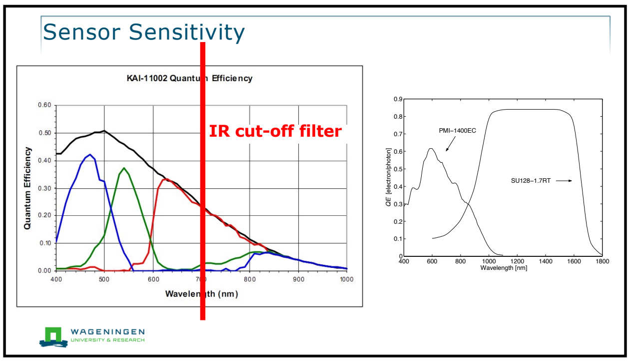
Sensor Sensitivity
IR cut-off filter
Quantum Efficiency
PMI-1400EC
SU128-1.7RT
Wavelength [nm]
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Implementation
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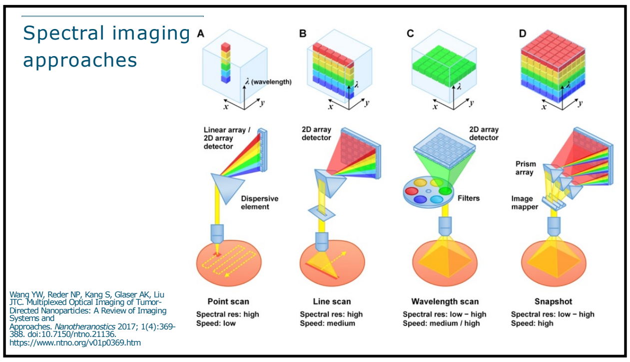
Spectral imaging approaches
A
B
C
D
λ (wavelength)
Linear array / 2D array detector
Dispersive element
2D array detector
2D array detector
Filters
Prism array
Image mapper
Point scan
Spectral res: high
Speed: low
Line scan
Spectral res: high
Speed: medium
Wavelength scan
Spectral res: low – high
Speed: medium / high
Snapshot
Spectral res: low – high
Speed: high
Wang YW, Reder NP, Kang S, Glaser AK, Liu JTC. Multiplexed Optical Imaging of Tumor-Directed Nanoparticles: A Review of Imaging Systems and Approaches. *Nanotheranostics* 2017; 1(4):369-388. doi:10.7150/ntno.21136. https://www.ntno.org/v01p0369.htm

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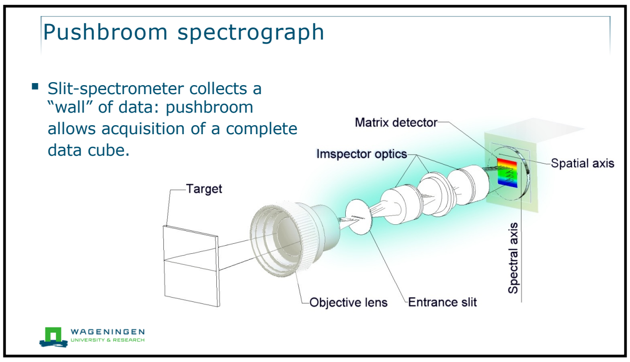
Pushbroom spectrograph
Slit-spectrometer collects a "wall" of data: pushbroom allows acquisition of a complete data cube.
Matrix detector
Inspector optics
Spatial axis
Target
Spectral axis
Objective lens
Entrance slit
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Some examples of cameras
specim IQ
reddot award 2018 winner
imec
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Latest developments 'snapshot cameras'
cubert
UV-VIS-NIR
Light Field-based Hyperspectral Video Camera
imec
SnapScan
SWIR
VNIR
specim IQ
reddot award 2018 winner

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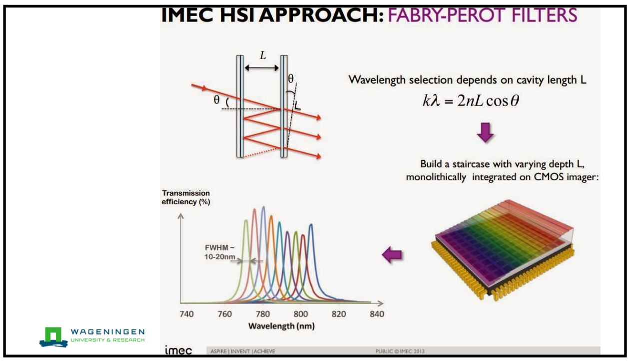
IMEC HSI APPROACH: FABRY-PEROT FILTERS
Wavelength selection depends on cavity length L
$k\lambda = 2nL\cos\theta$
Build a staircase with varying depth L, monolithically integrated on CMOS imager:
Transmission efficiency (%)
FWHM ~ 10-20nm
740 760 780 800 820 840
Wavelength (nm)
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imec

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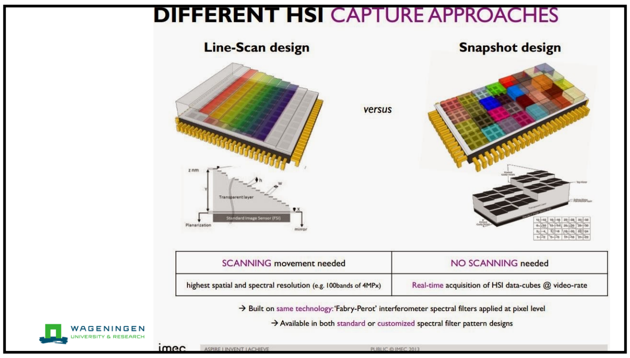
DIFFERENT HSI CAPTURE APPROACHES
Line-Scan design
versus
Snapshot design
Transparent layer
Standard Image Sensor (FSI)
Planarization
mirror
SCANNING movement needed
NO SCANNING needed
highest spatial and spectral resolution (e.g. 100bands of 4MPx)
Real-time acquisition of HSI data-cubes @ video-rate
→ Built on same technology: 'Fabry-Perot' interferometer spectral filters applied at pixel level
→ Available in both standard or customized spectral filter pattern designs
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imec
ASPIRE | INVENT | ACHIEVE
PUBLIC © IMEC 2013

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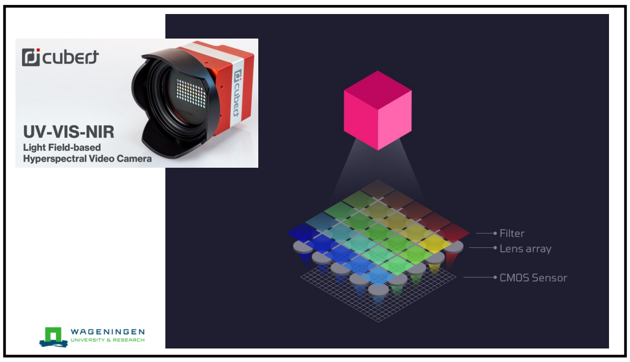
cubert
UV-VIS-NIR
Light Field-based
Hyperspectral Video Camera
Filter
Lens array
CMOS Sensor
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Exercise 1
Google Colab

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Calibration / normalisation

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Calculation spectral reflectance

$$R_\lambda = \frac{I_\lambda - B}{W_\lambda - B}$$

- R_λ - real reflection at wavelength λ
- I_λ - original measured reflection
- W_λ - spectral radiation of the illuminant
- B is the black reference
- This calculation/measurement of W_λ and B, needs to be done at a regular interval, at least as fast as the drift in the disturbing factors.

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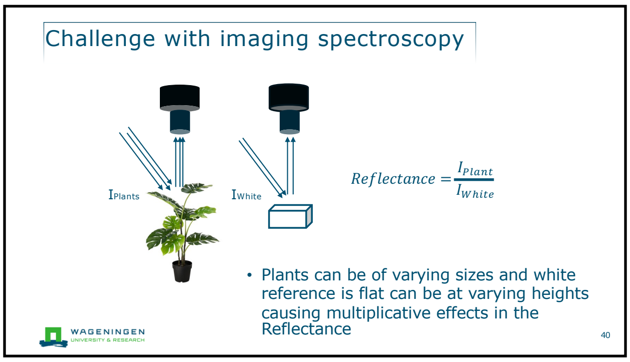
Challenge with imaging spectroscopy
IPlants
IWhite
$Reflectance = \frac{I_{Plant}}{I_{White}}$
• Plants can be of varying sizes and white reference is flat can be at varying heights causing multiplicative effects in the Reflectance
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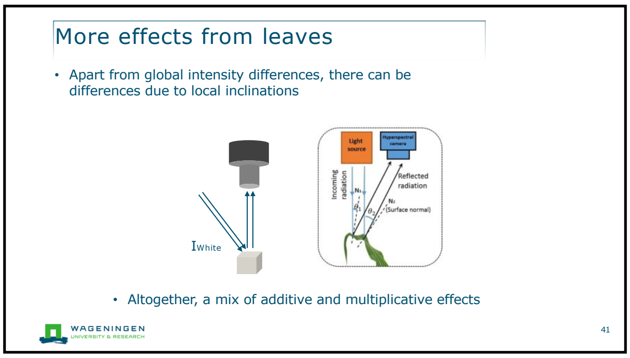
More effects from leaves
• Apart from global intensity differences, there can be differences due to local inclinations
IWhite
Light source
Incoming radiation
Reflected radiation
• Altogether, a mix of additive and multiplicative effects
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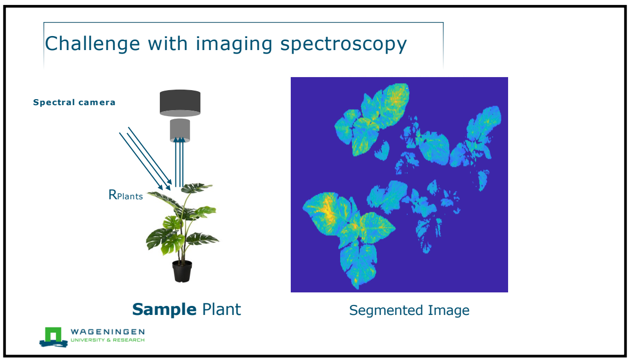
Challenge with imaging spectroscopy
Spectral camera
RPlants
Sample Plant
Segmented Image
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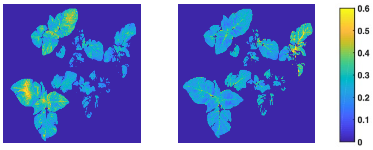
A solution is chemometric normalisation



Before ***After***

- *Mishra, Puneet, et al. "Close-range hyperspectral imaging of whole plants for digital phenotyping: Recent applications and illumination correction approaches." Computers and Electronics in Agriculture 178 (**2020**): 105780.*
- *Mishra, Puneet, et al. "Utilising **variable sorting for normalisation** to correct illumination effects in close-range spectral images of potato plants." biosystems engineering 197 (**2020**): 318-323.*

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Projects

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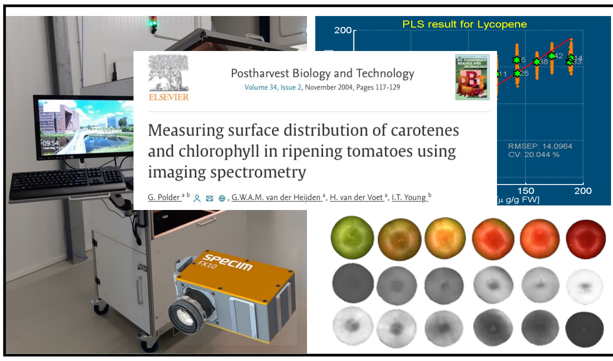

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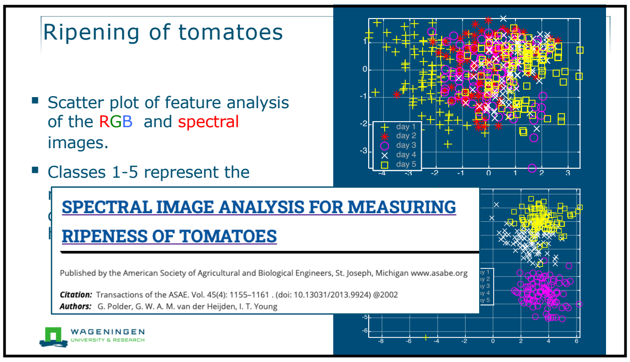
Ripening of tomatoes
Scatter plot of feature analysis of the RGB and spectral images.
Classes 1-5 represent the
day 1
day 2
day 3
day 4
day 5
SPECTRAL IMAGE ANALYSIS FOR MEASURING RIPENESS OF TOMATOES
Published by the American Society of Agricultural and Biological Engineers, St. Joseph, Michigan www.asabe.org
Citation: Transactions of the ASAE. Vol. 45(4): 1155-1161 . (doi: 10.13031/2013.9924) @2002
Authors: G. Polder, G. W. A. M. van der Heijden, I. T. Young
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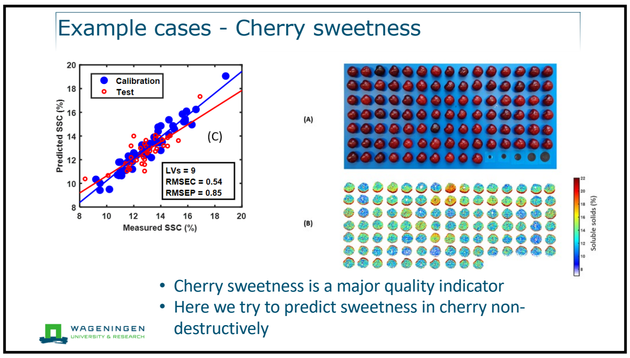
Example cases - Cherry sweetness
Calibration
Test
Predicted SSC (%)
Measured SSC (%)
(C)
LVs = 9
RMSEC = 0.54
RMSEP = 0.85
(A)
(B)
Soluble solids (%)
• Cherry sweetness is a major quality indicator
• Here we try to predict sweetness in cherry non-destructively
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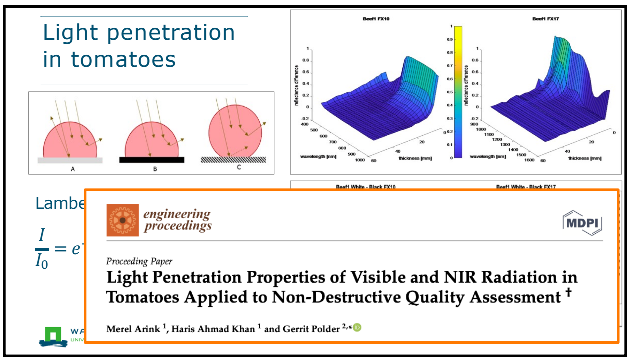
Light penetration in tomatoes
A
B
C
Lambe
engineering proceedings
MDPI
Proceeding Paper
Light Penetration Properties of Visible and NIR Radiation in Tomatoes Applied to Non-Destructive Quality Assessment †
Merel Arink 1, Haris Ahmad Khan 1 and Gerrit Polder 2,*

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Salinity stress in tobacco leaves

- Salinity stress severely affects plant growth and causes significant yield reductions
- It commonly occurs in arid and semi-arid zones naturally or because of anthropogenic influences such as irrigation with reclaimed water

- *Puneet Mishra, et al. "Close range hyperspectral imaging for mapping salinity stress induced by Red Sea water irrigation in Tobacco leaves." HSI 2015 conference, Coventry, UK.*

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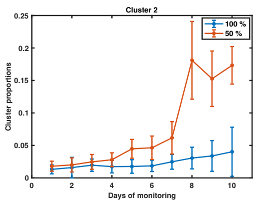
Drought stress in Arabidopsis plants with portable imaging spectroscopy



- *Mishra, Puneet, et al. "Early detection of drought stress in Arabidopsis thaliana utilsing a portable hyperspectral imaging setup." 2019 10th Workshop on Hyperspectral Imaging and Signal Processing: Evolution in Remote Sensing (WHISPERS). IEEE, 2019.*

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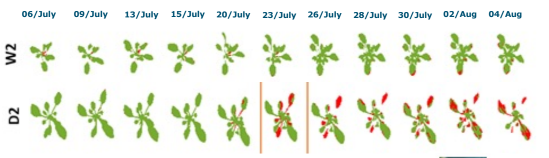
Drought stress detection in arabidopsis in digital phenotyping framework



האוניברסיטה העברית בירושלים
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Monitoring crop status - goal

- Crop growers need information on status of the leaves before they are removed during crop cultivation.
- Currently this can only be done using leaf samples send to an external laboratory.
- Can spectral imaging be used for measuring leaf and fruit compounds non-destructively?

Anja Dieleman
Esther Meinen
Jeroen van Arkel
Kees Weerheim

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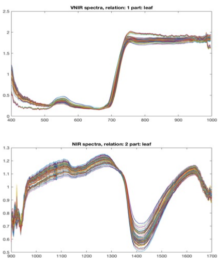
Monitoring crop status - experiment

- VNIR HSI, Specim V10e, 400-1000nm
- NIR HSI, Specim N17, 900-1700nm
- 412 leaf samples
- 200 fruits
- Supervised foreground/background segmentation
- Average spectrum per sample
- Reference measures
- Partial Least Square (PLS) regression, using leave one out cross validation.



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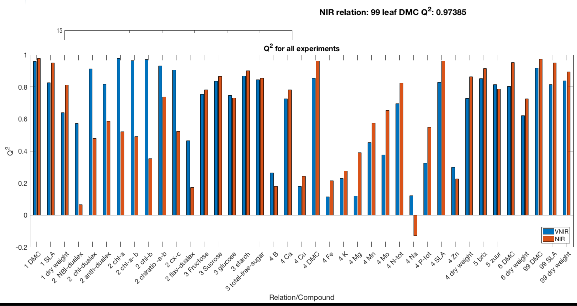
Monitoring crop status - results



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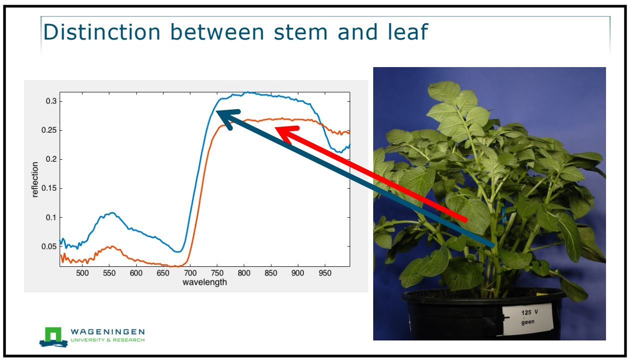
Distinction between stem and leaf
reflection
wavelength
0.3
0.25
0.2
0.15
0.1
0.05
500 550 600 650 700 750 800 850 900 950
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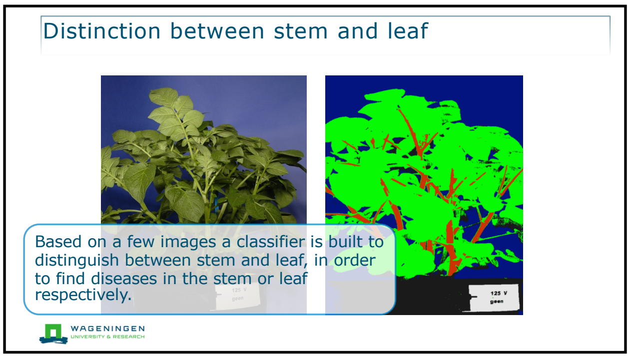
Distinction between stem and leaf
Based on a few images a classifier is built to distinguish between stem and leaf, in order to find diseases in the stem or leaf respectively.
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SPECIM
Detection of virus and bacterial diseases in seed potatoes in the open field
Toon Tielen

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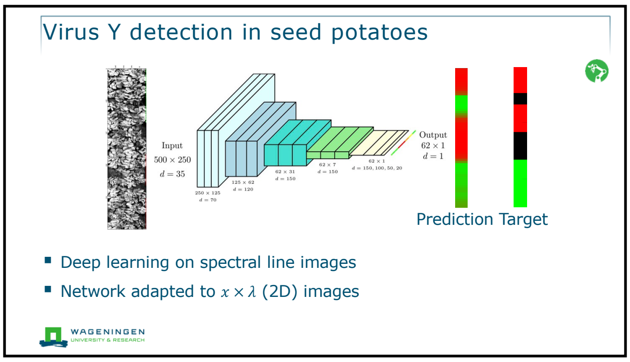
Virus Y detection in seed potatoes
Input
500 × 250
d = 35
Output
62 × 1
d = 1
Prediction Target
Deep learning on spectral line images
Network adapted to $x \times \lambda$ (2D) images
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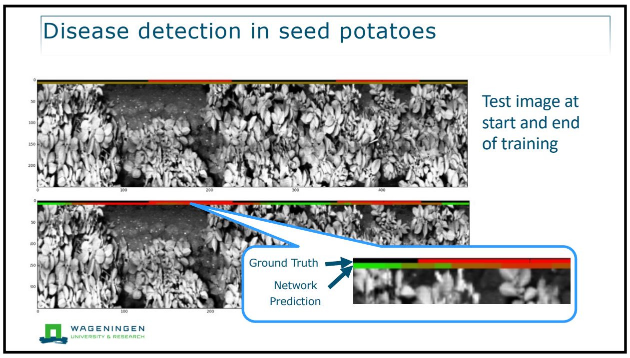
Disease detection in seed potatoes
Test image at start and end of training
Ground Truth
Network Prediction
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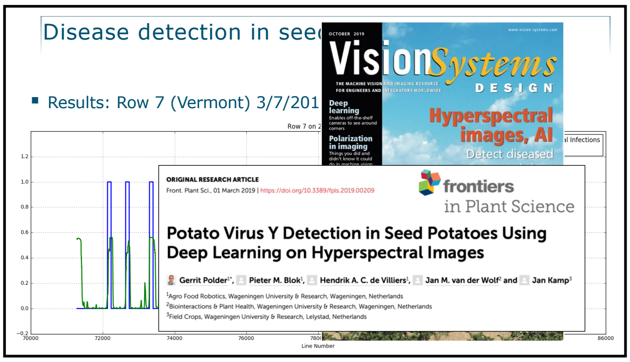
Disease detection in see
Results: Row 7 (Vermont) 3/7/201
Vision Systems
DESIGN
Hyperspectral images, AI
Detect diseased
Deep learning
Polarization in imaging
ORIGINAL RESEARCH ARTICLE
frontiers in Plant Science
Potato Virus Y Detection in Seed Potatoes Using Deep Learning on Hyperspectral Images
Gerrit Polder, Pieter M. Blok, Hendrik A. C. de Villiers, Jan M. van der Wolf and Jan Kamp
Line Number

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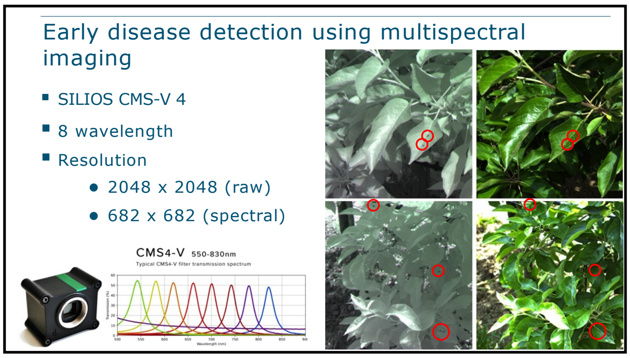
Early disease detection using multispectral imaging
SILIOS CMS-V 4
8 wavelength
Resolution
2048 x 2048 (raw)
682 x 682 (spectral)
CMS4-V 550-830nm

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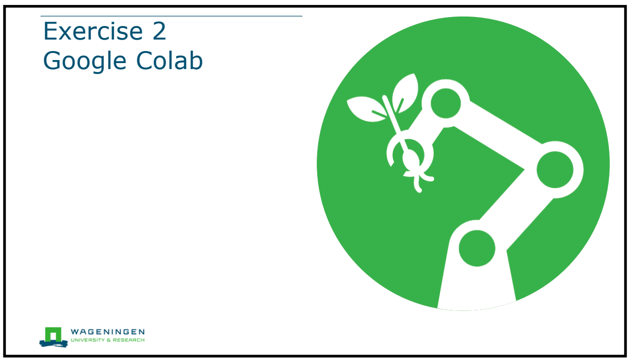
Exercise 2
Google Colab
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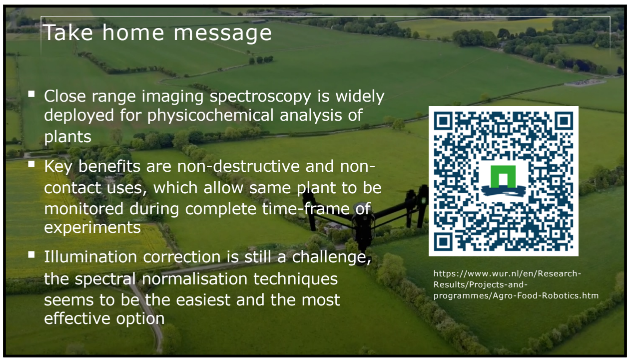
Take home message
Close range imaging spectroscopy is widely deployed for physicochemical analysis of plants
Key benefits are non-destructive and non-contact uses, which allow same plant to be monitored during complete time-frame of experiments
Illumination correction is still a challenge, the spectral normalisation techniques seems to be the easiest and the most effective option
https://www.wur.nl/en/Research-Results/Projects-and-programmes/Agro-Food-Robotics.htm

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Take home message
From my wide range of experience and application I can recommend that imaging spectroscopy is a very useful tool for plant analysis and particularly for plant phenotyping
Future trend will be related to combining imaging spectroscopy, chemometric knowledge and artificial intelligence to mine the enormous data generated by imaging spectroscopy.
https://www.wur.nl/en/Research-Results/Projects-and-programmes/Agro-Food-Robotics.htm

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