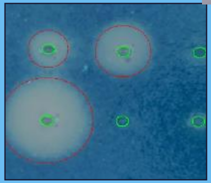
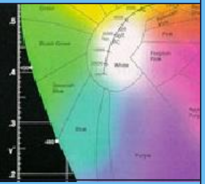
Spectral image analysis of seeds, plants, and pathogens with the VideometerLab

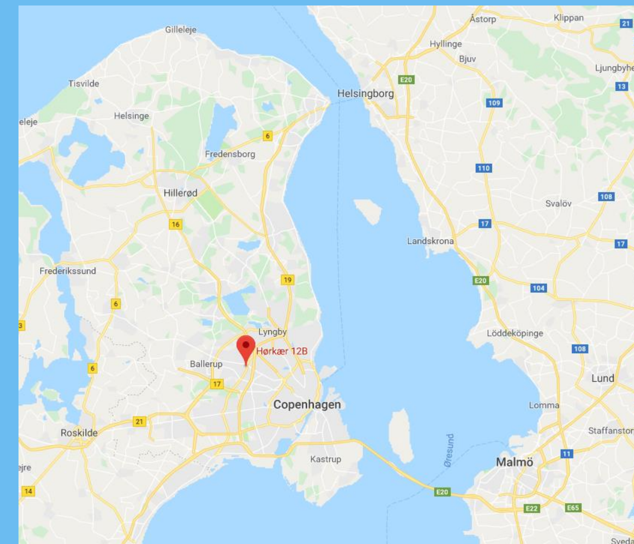
Jens Michael Carstensen
CEO, Videometer A/S





Videometer

- Spectral imaging company
- Founded 1999
- App. 600 imaging R&D projects since 2000
- In-line 24/7 spectral imaging since 2002
- Markets R&D projects, instruments, and software
- Patented technology
- Based in Herlev, Denmark



Imaging

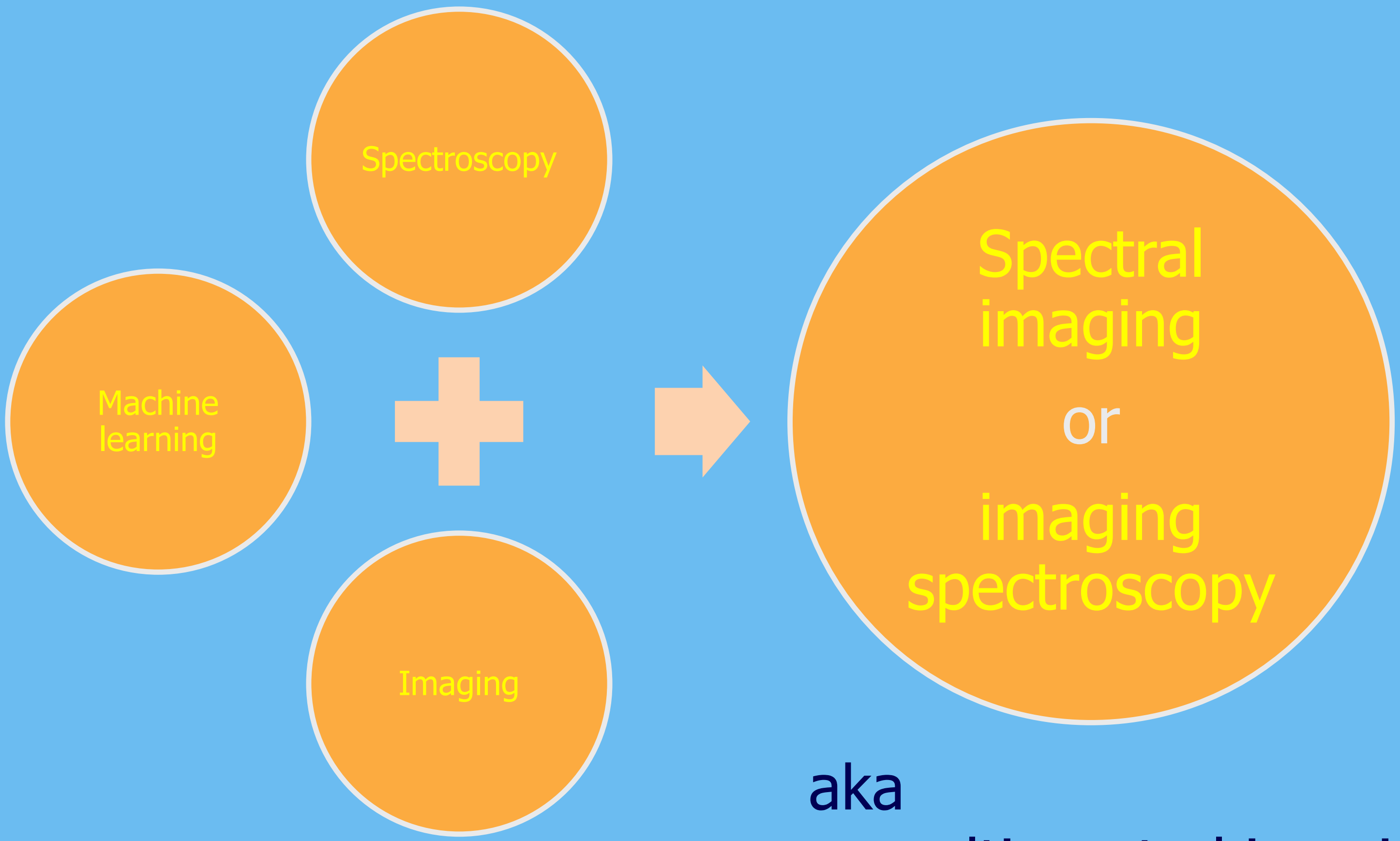
Is imaging just about taking a picture and then find the right app to calculate some features?

Phenotype this

Appearance = Chemistry X Physics X Environment X Illumination

Chromatic Adaptation Demo

Spectral imaging



aka

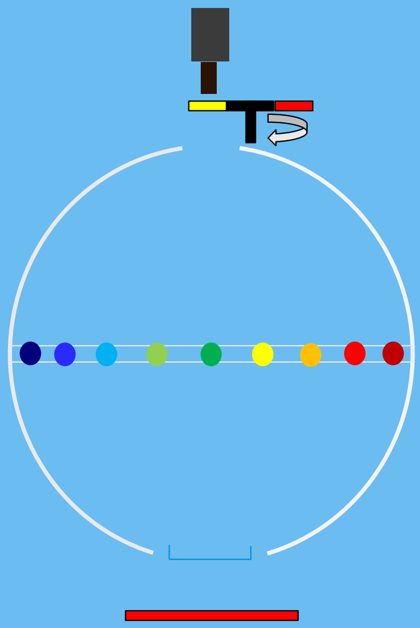
- multispectral imaging
- hyperspectral imaging

Primary attributes for phenotyping and QC

- Color (surface chemistry)
- Shape
- Anatomical traits
- Topographical texture
- Spectral texture
- Gloss
- Shape
- Size
- Position and orientation
- Count

Video

Videometer band-sequential spectral imaging



- LEDs: Stable, durable, large selection, rapidly developing technology
- Up to 20 different high-resolution bands acquired sequentially in 0.5-1.5 seconds depending on camera
- May be combined with emission filters, backlight, and darkfield illuminant

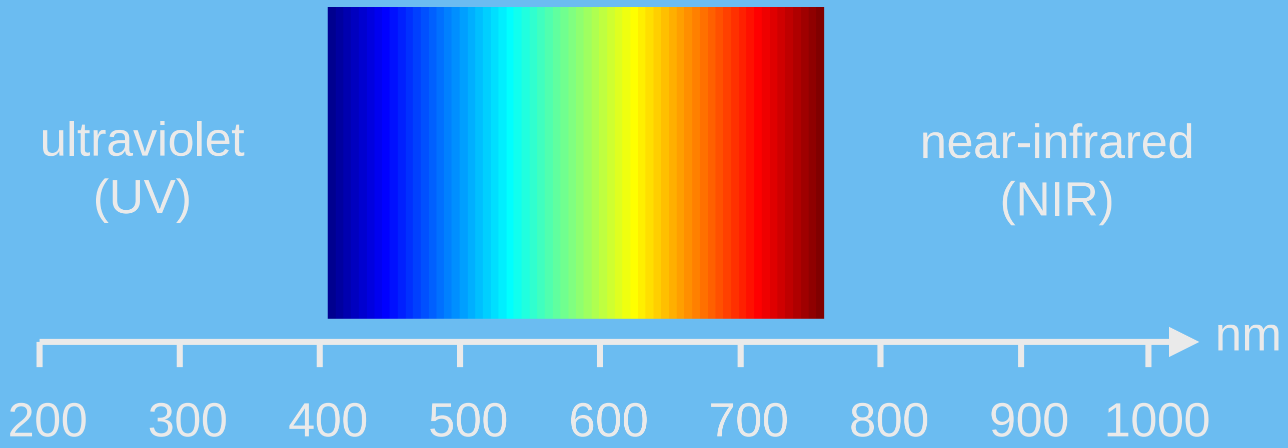
Combined reflectance spectral imaging and fluorescence spectral imaging!

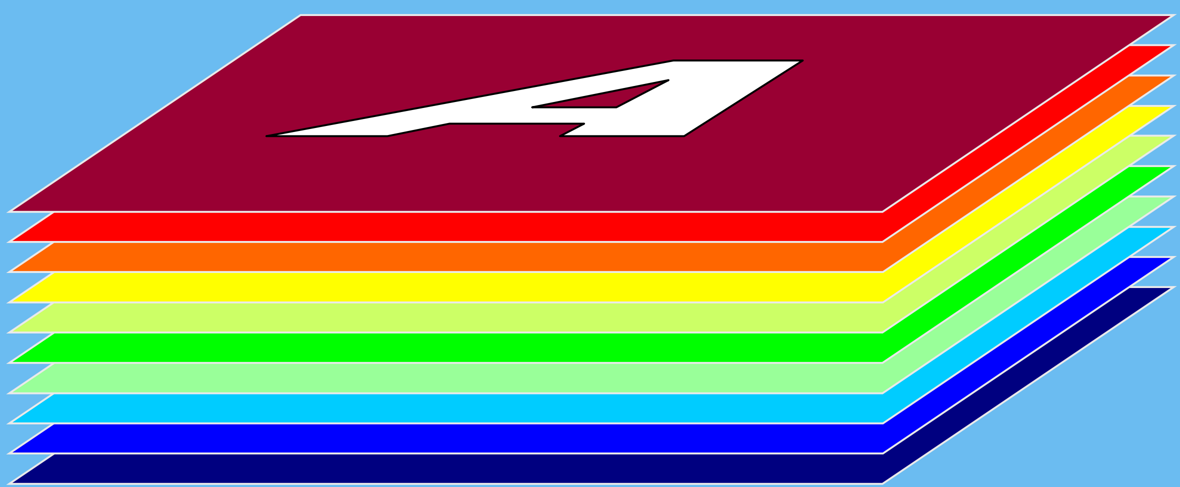
VideometerLab 4

Laboratory device for spectral imaging

- 19-20 spectral bands in the range 365 nm to 1000 nm
- 2192 × 2192 pixels per band, 41 µm (2704 x 2704 high-res option, 33 µm)
- Very homogeneous and diffuse illumination
- Strobed LED light source
- 10 seconds per sample including handling
- Optional backlight strobe
- Optional fluorescence bands
- Software for calibration, acquisition, and analysis
- Patented technology

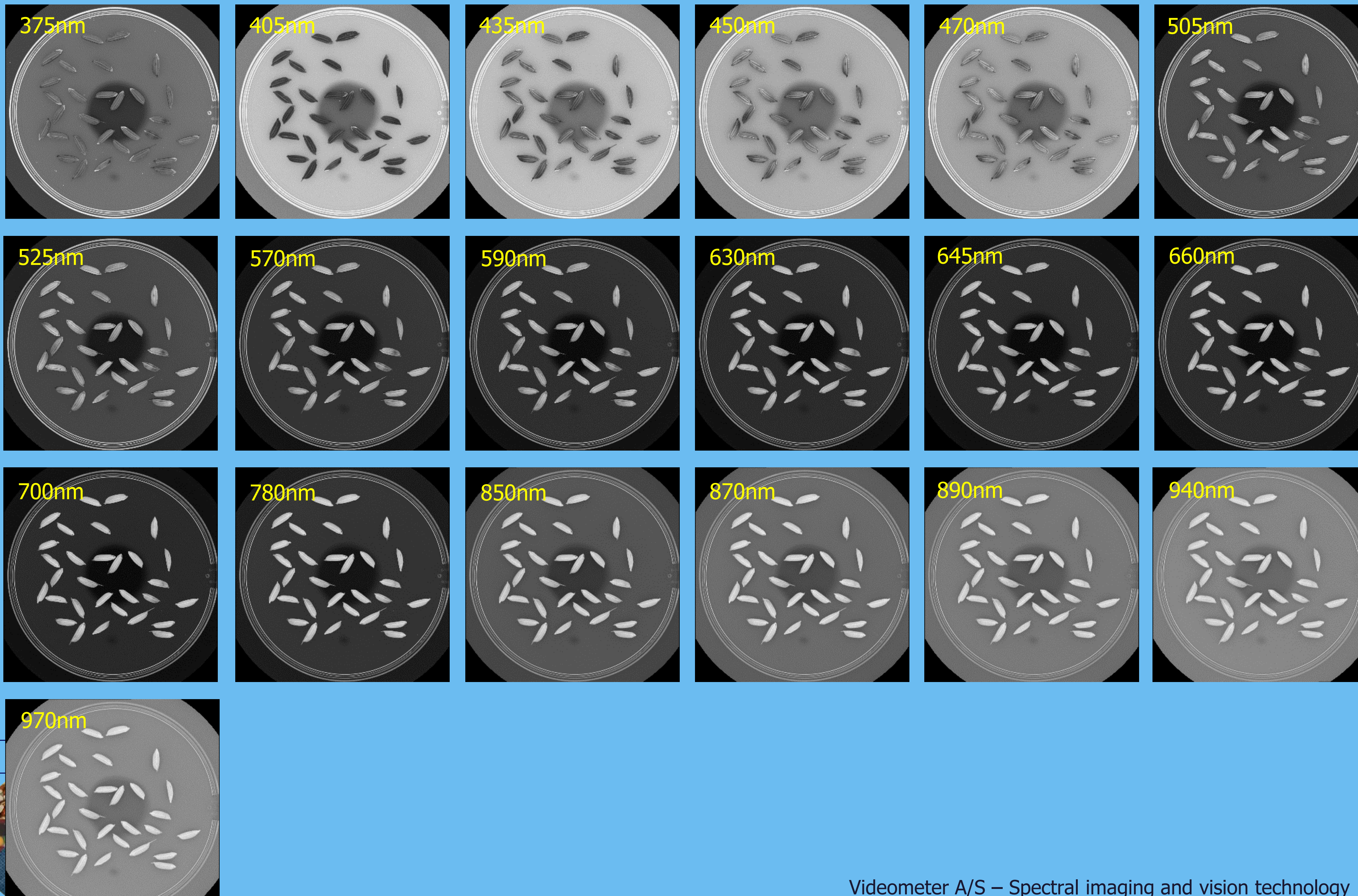
Spectral Imaging





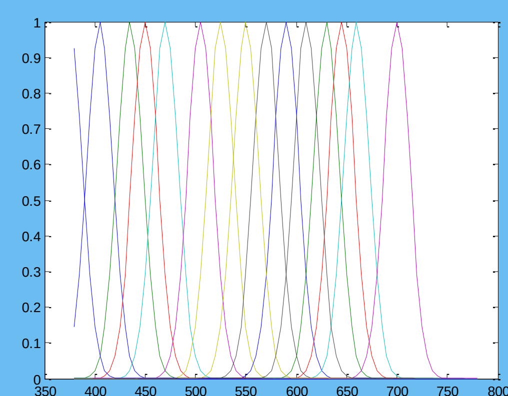
N images
obtained at
N specific
wavelengths

Image example (Rice *Oryza sativa* L.)

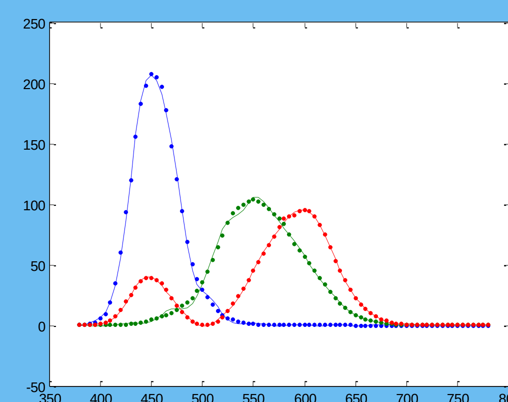


Simulation of illuminants

D65 D50 F11



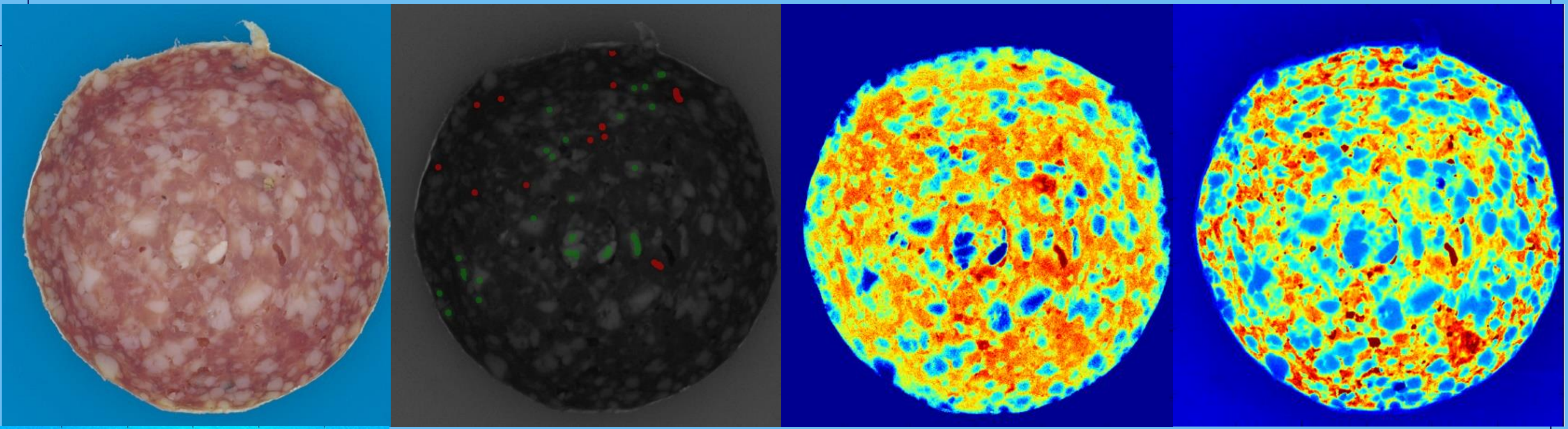
Normalized LED spectra

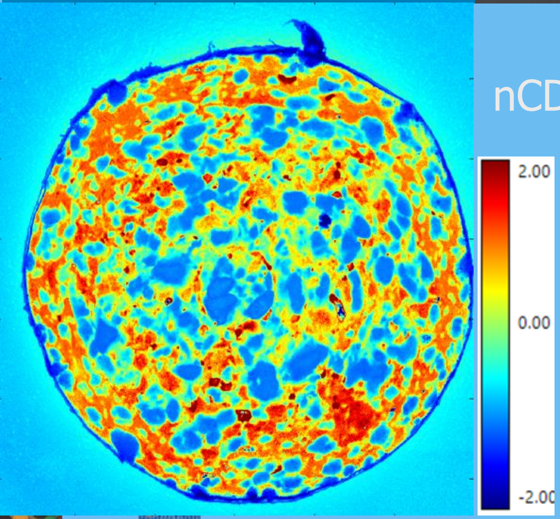


Linear model of CIE XYZ under D65

Canonical discriminants (CDA/nCDA)

sRGB | Training sets | Raw CDA (R=6.5) | nCDA (R=53.6)



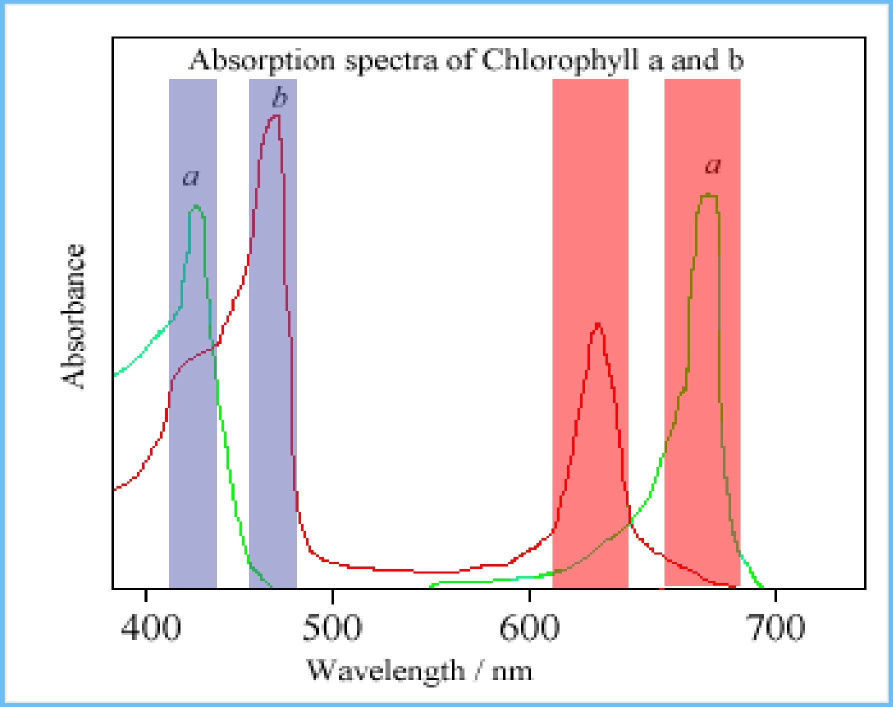


nCDA w/basis expansion(R=97.4)

$$R_{\max} = \max_{\mathbf{a}} R(\mathbf{a}) = \frac{\mathbf{a}^{T}\mathbf{A}\mathbf{a}}{\mathbf{a}^{T}\mathbf{W}\mathbf{a}}$$

Advantage of multiple wavelengths

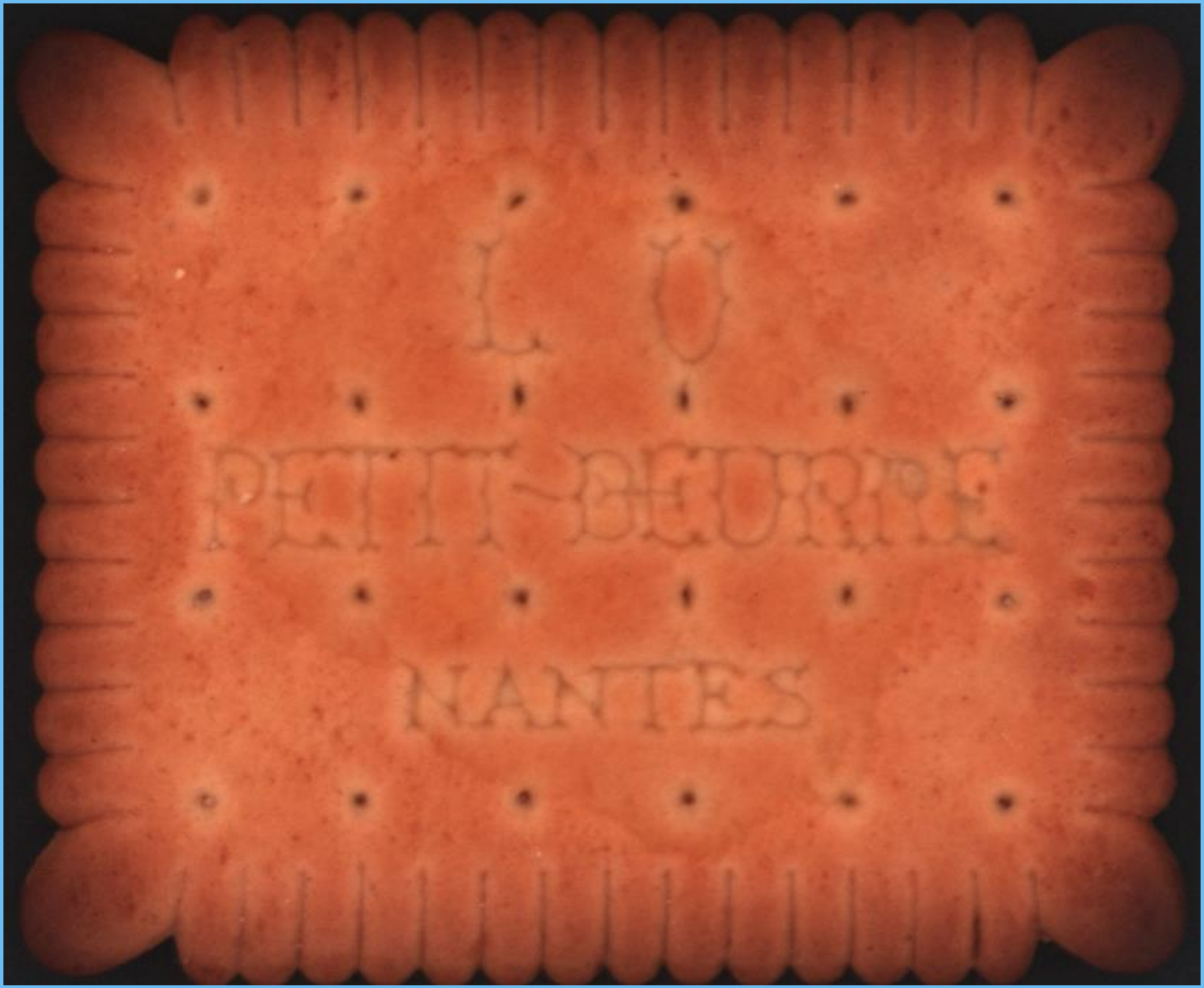
Using specific wavelengths for imaging, chlorophyll a and b can be distinguished *



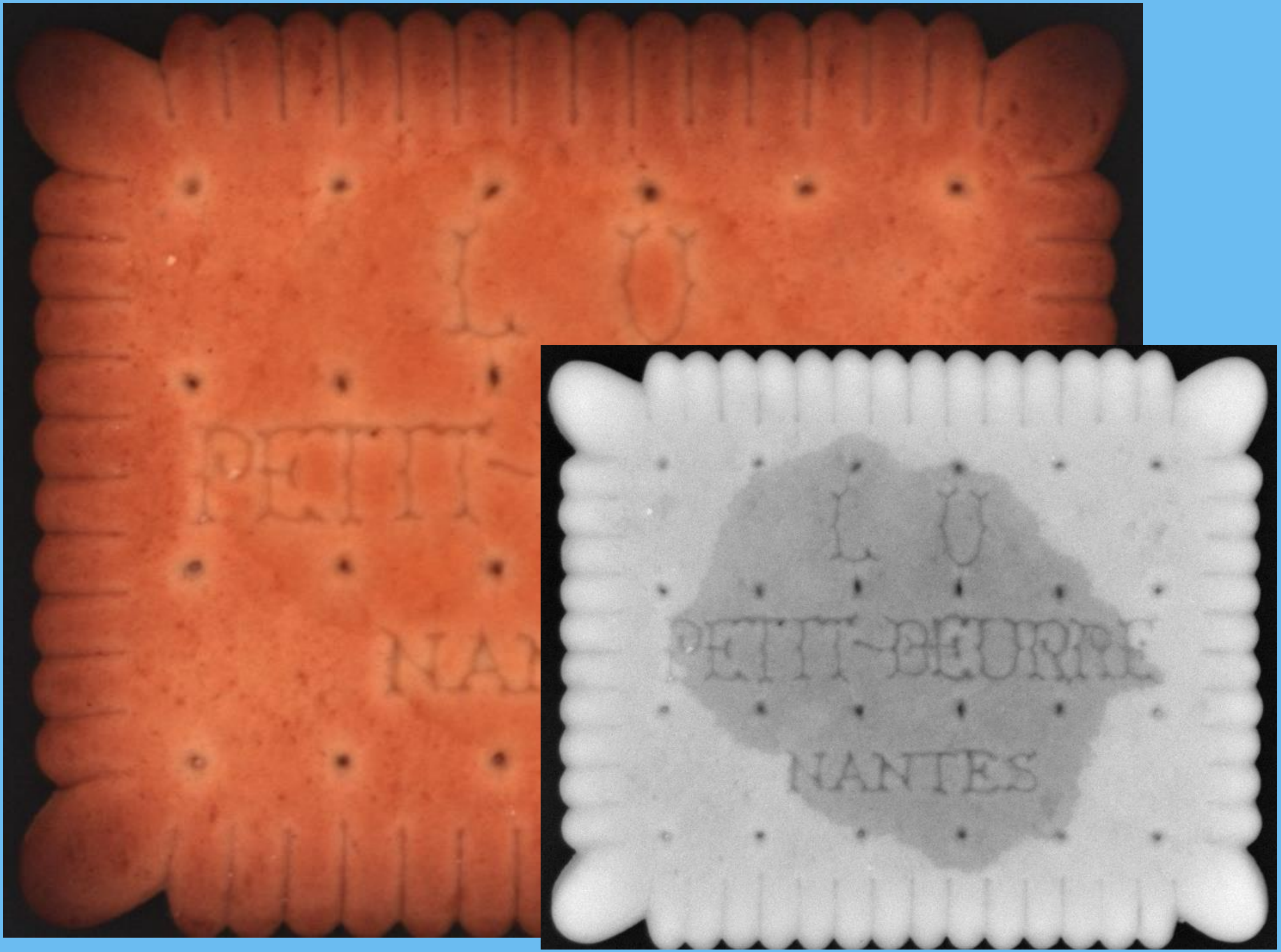
Chl.A	low	high	high	low
Chl.B	high	low	low	high

* Pan et al. (2015) http://www.nature.com/articles/srep11108#f6

Biscuit with wet spot



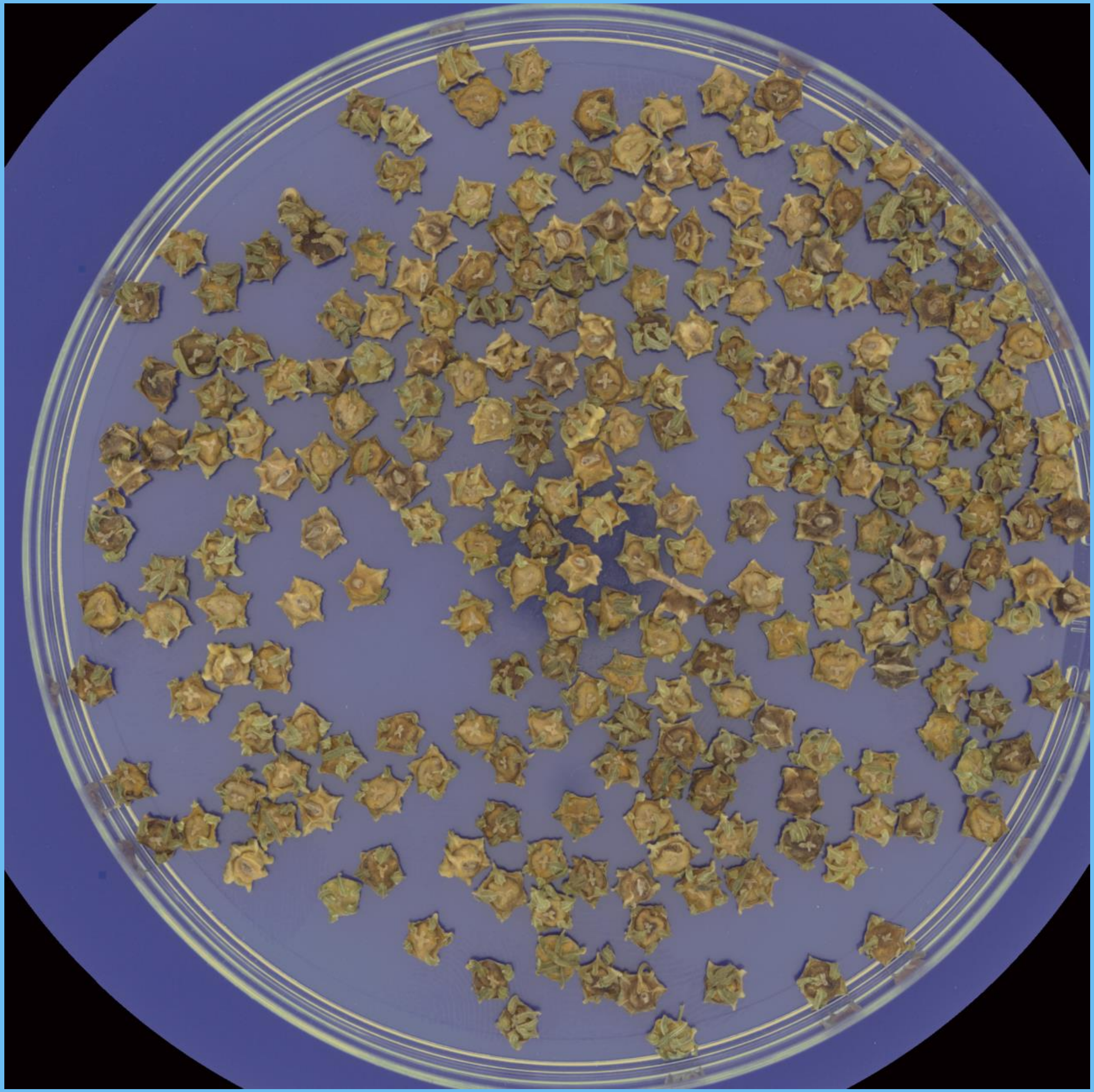
Moisture detection on biscuit



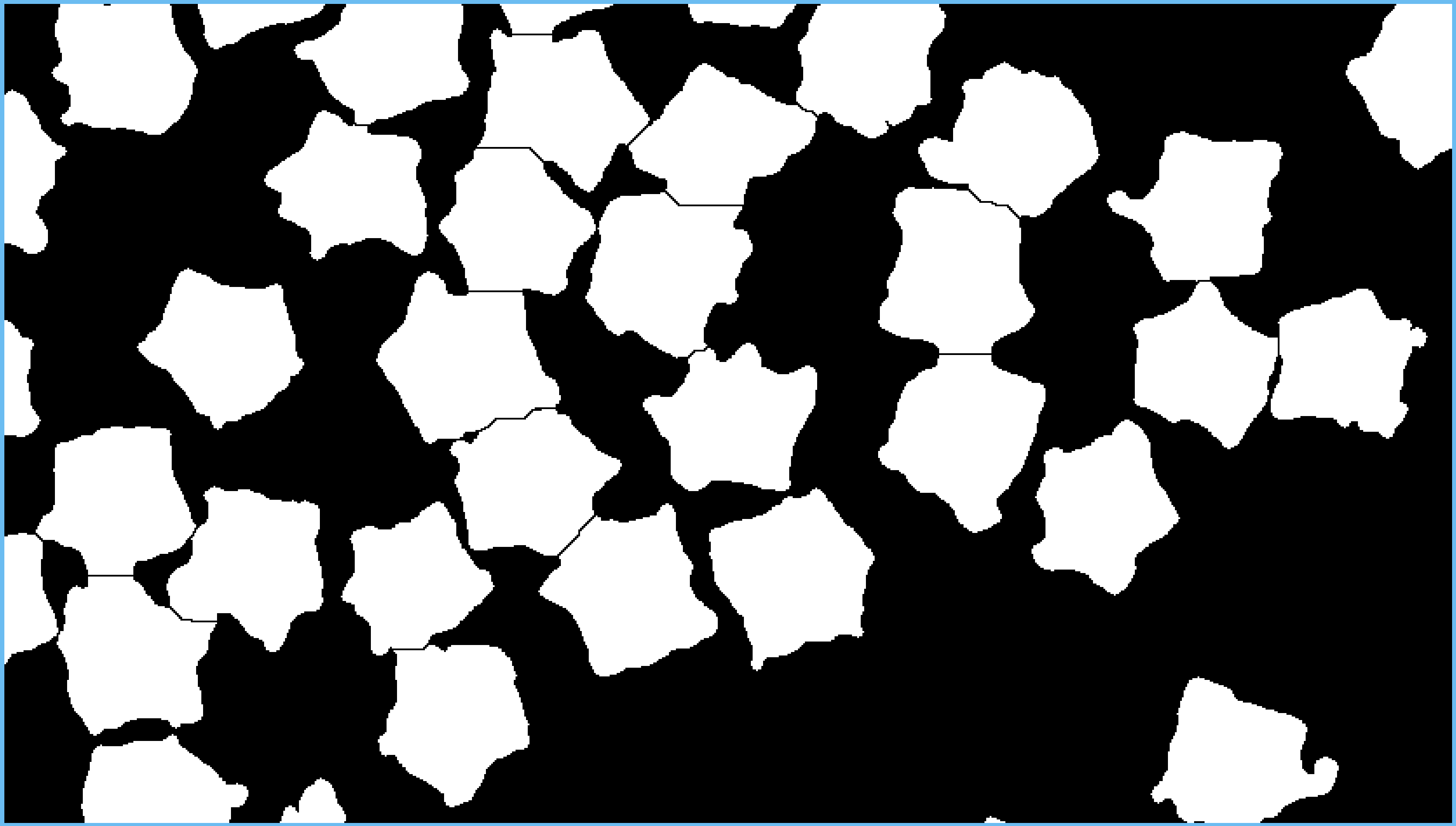
Videometer throughout the seed/grain chain

- Breeding and genetic resources
  - Screening, phenotyping, ploidy, genebank management (off-type, phenotype query)
- Seed technology
  - Seed coating, seed priming, seed pelleting, seed disinfection, seedborne disease control
- Sowing
  - Germination, vigor, hydration, root and shoot analysis
- Growing
  - Field and greenhouse phenotyping, stressors, resistance
- Harvesting
  - Maturity assessment, Preharvest sprouting, Combine harvester control
- Trading
  - Product appraisal, OEM product for receival stations: EyeFoss (wheat, barley, rice etc.)
- Cleaning
  - Purity, broken, high value seed sorting, self-adjusting cleaning machines
- Refining
  - Milling, mixing, malting

Sugar beet seeds

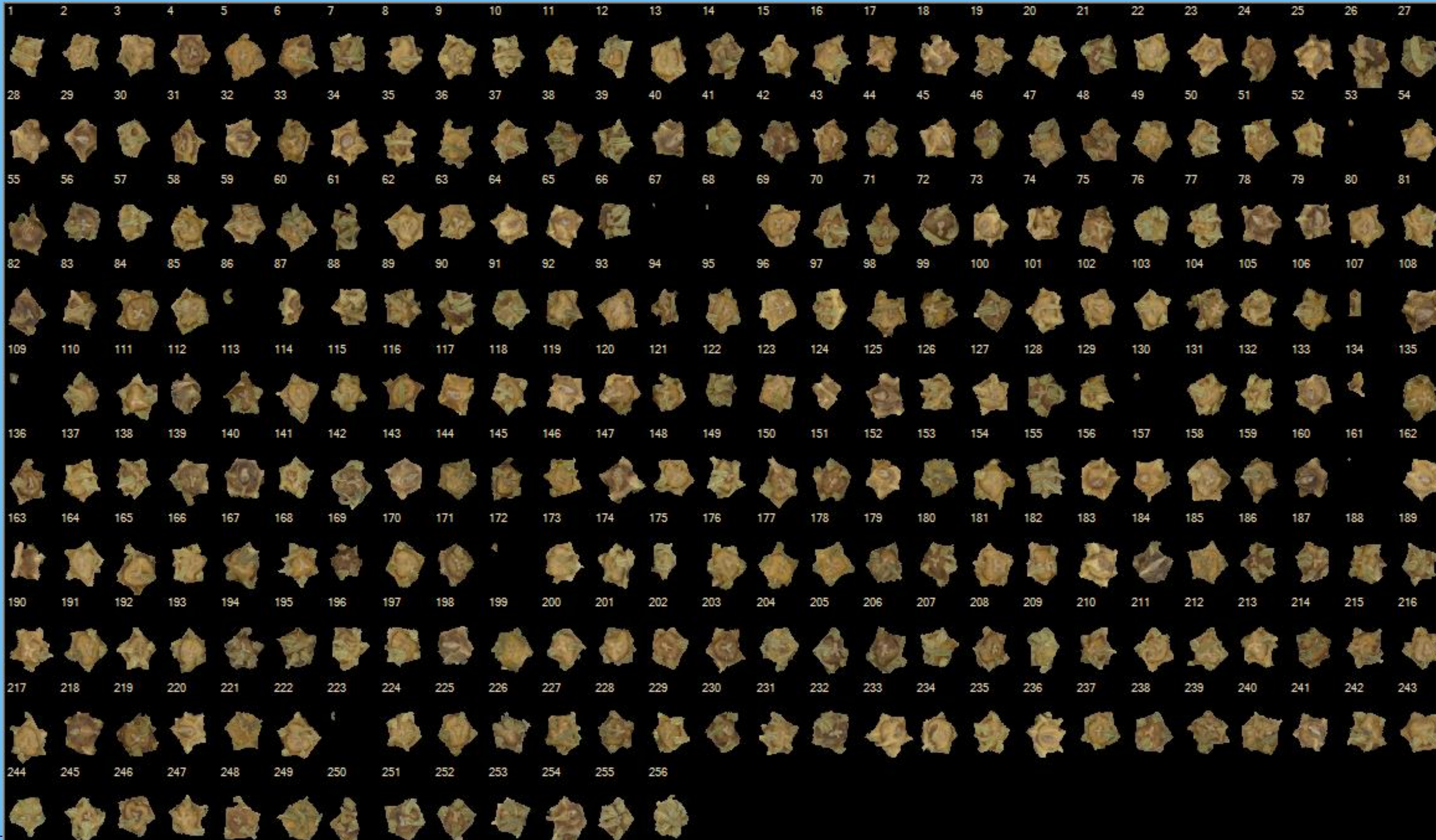


Sugar beet seeds segmented

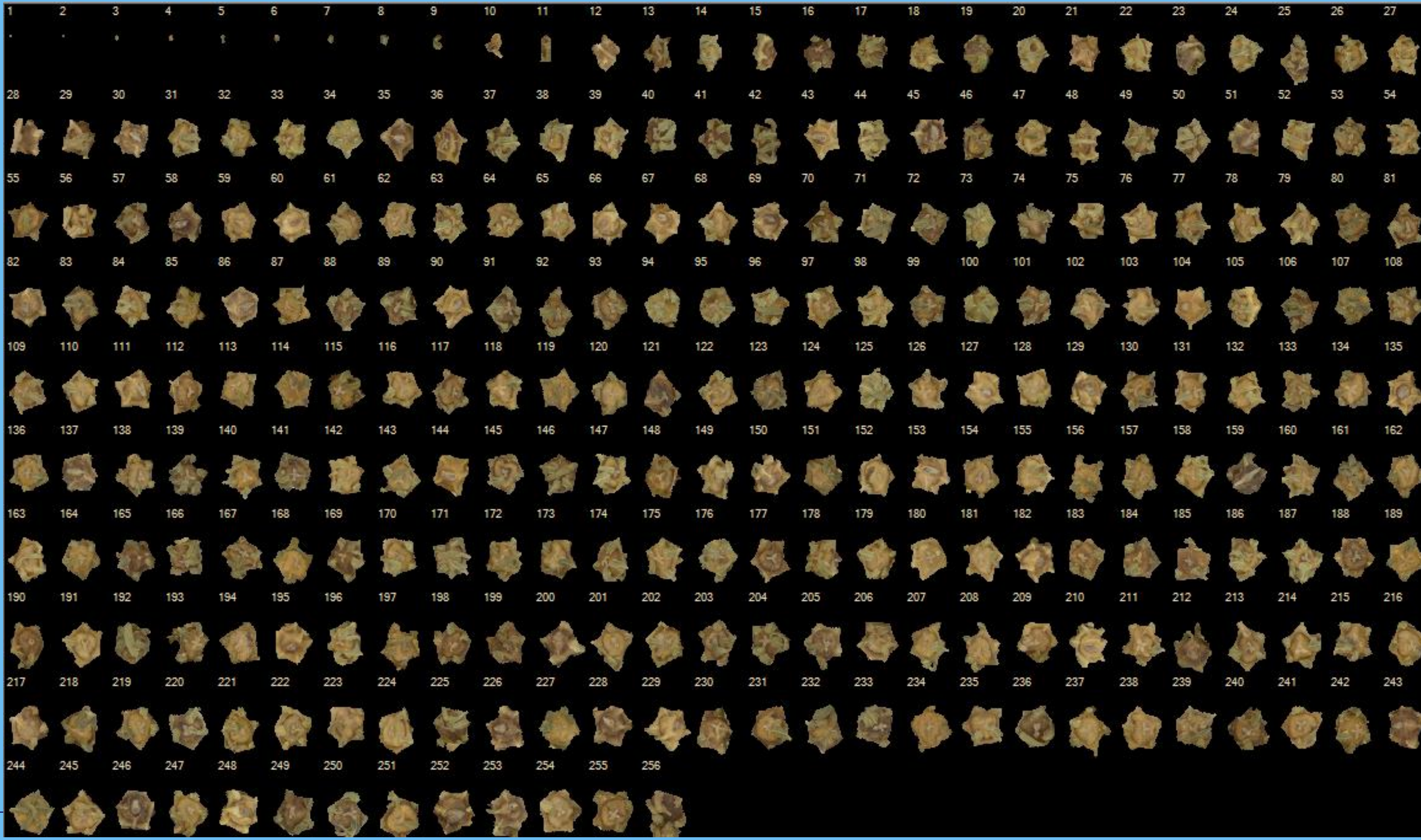


Videometer

Sugar beet seeds aligned

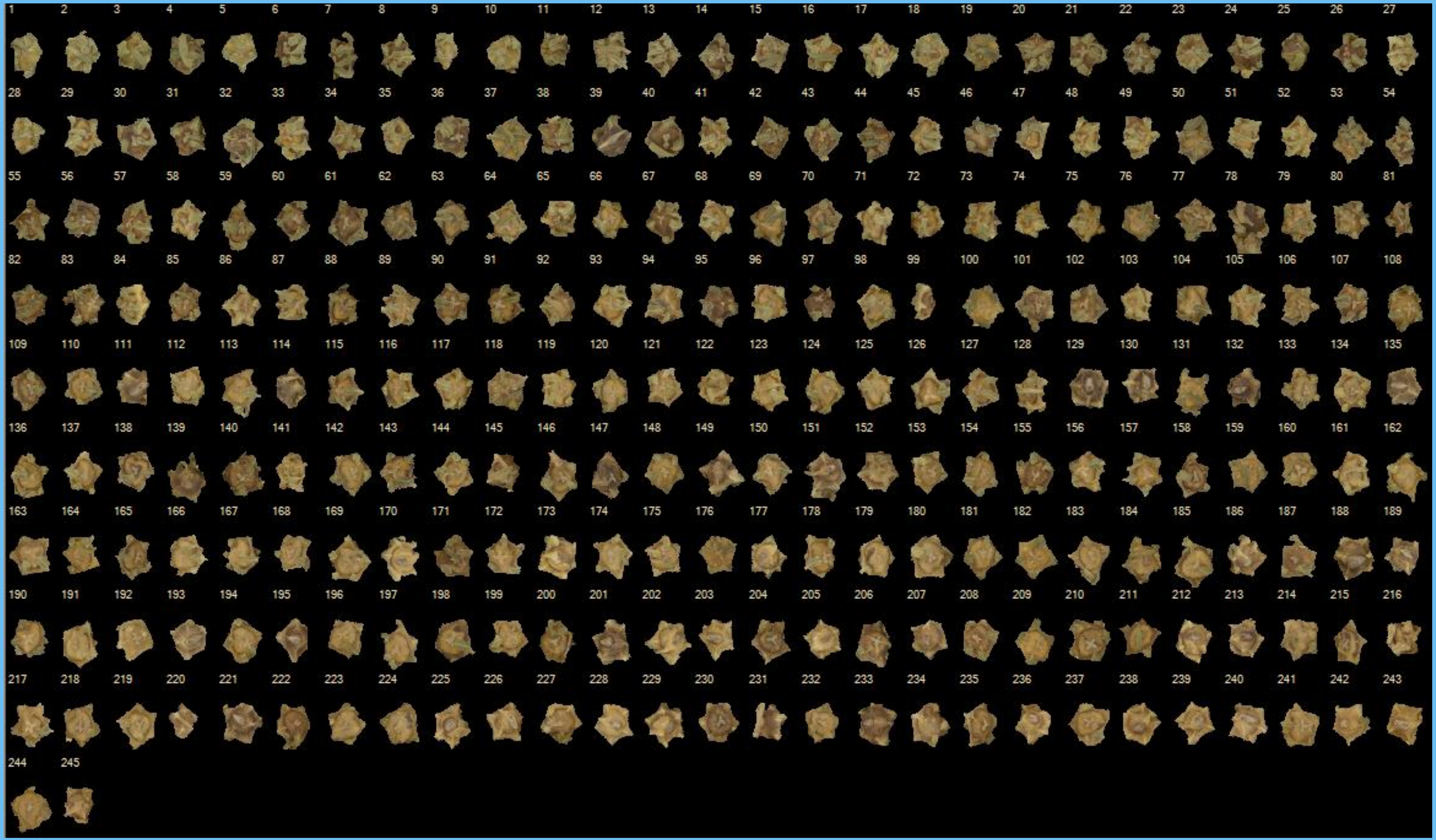


Seeds sorted by area



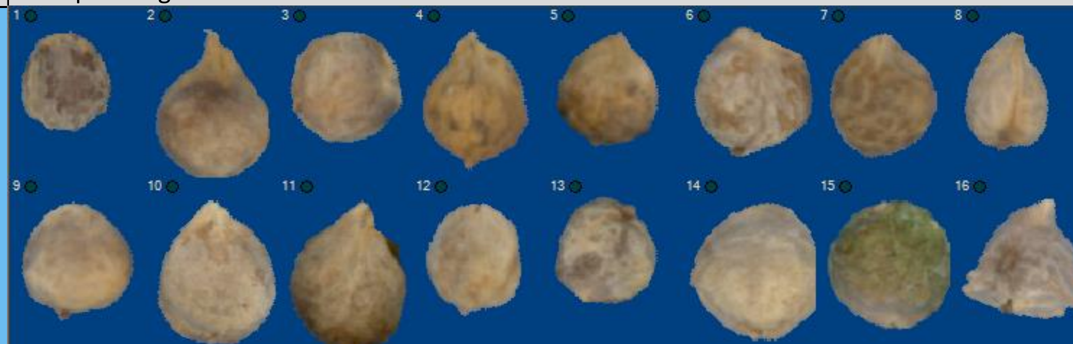
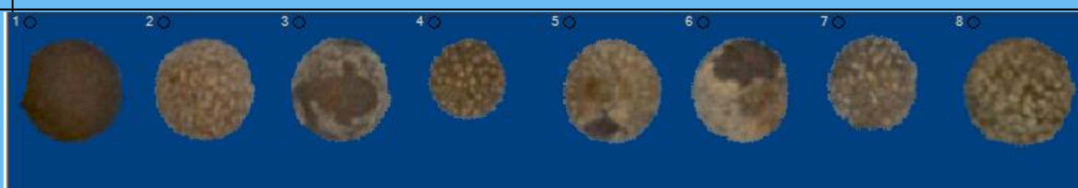
Fines can be removed

Seeds sorted by chlorophyll

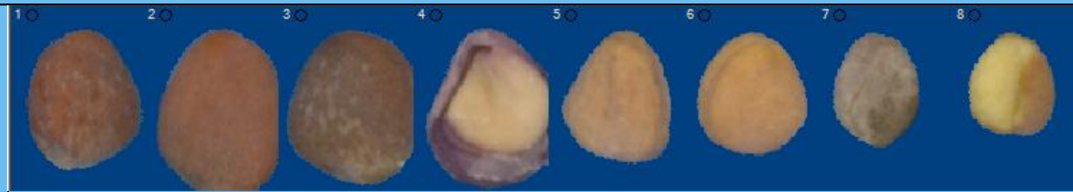
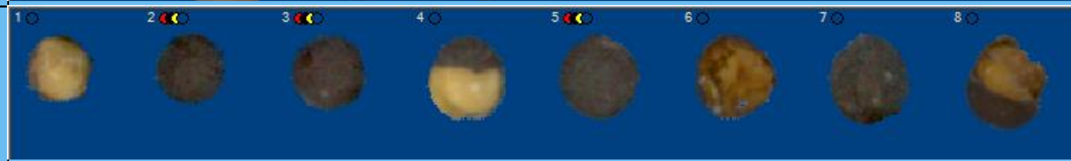
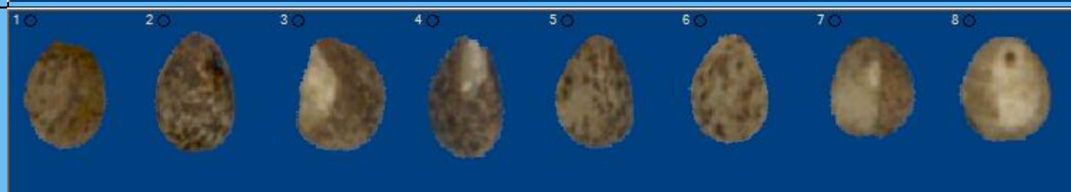


SEED PURITY

Purity analysis of spinach samples

Class	Example Images	Features	Sensitivity
Spinach	 	Color, shape, texture	99.9%
Cleavers / Galium aparine		Primarily shape and texture	99.5%
Black bindweed/ Polygonum convolvulus		Texture, shape and color for	99.7%

Purity analysis of spinach samples

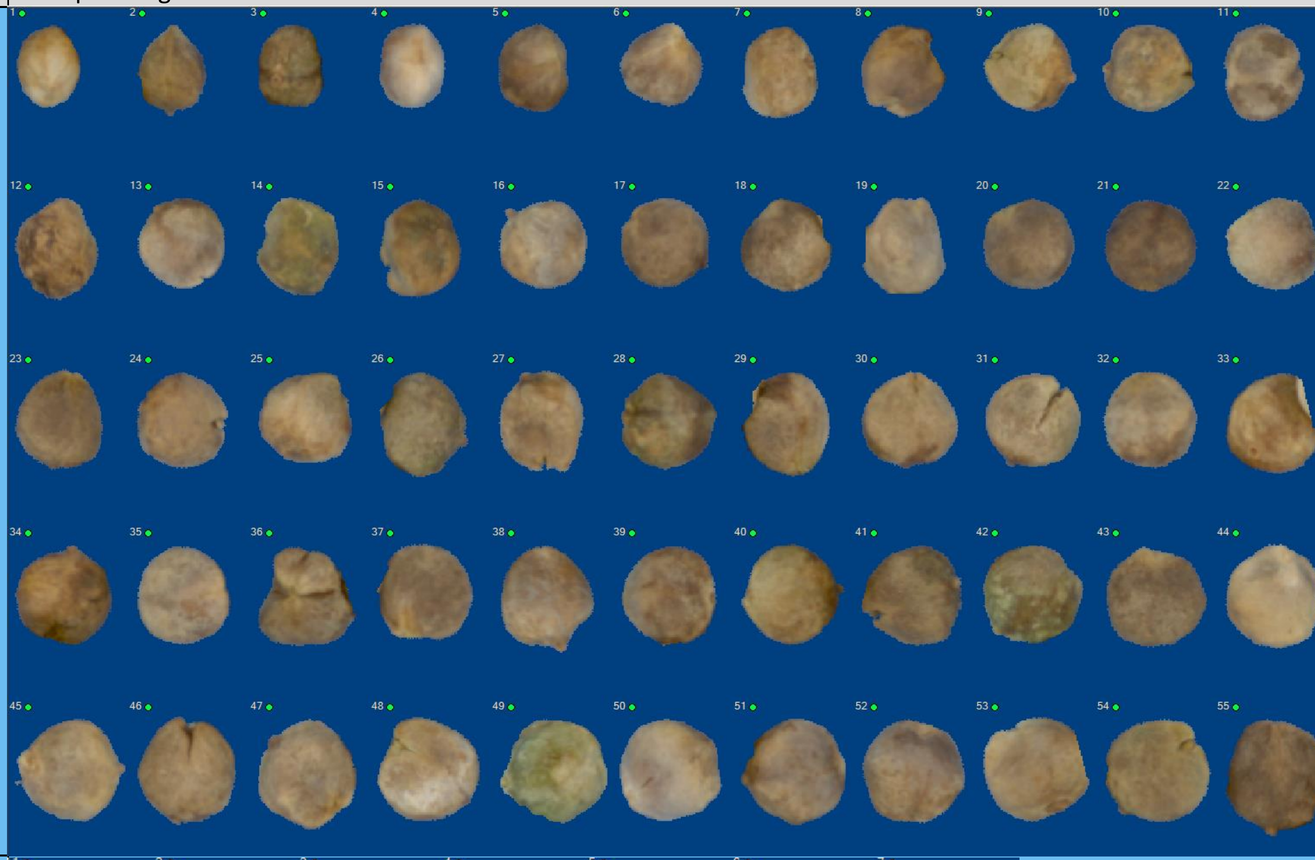
Radish/ Raphanus radicula		Color and smoothnes of surface.	97.6%
Rapeseed/ Brassica napus		Color and shape	97.9%
Hemp-nettle/ Galeopsis		Texture and color	98.2%
Cereal		Shape, size, color of broken kernel and presence of furrow on ventral side	99.7%

Second level attributes - Seed classification

Classifier performance on test set with 57115 seeds

Reference \ Predicted	Cereal	Spinach	Cleavers	Black bindweed	Radish	Rapeseed	Hemp-nettle	Total	Error
Cereal	99.7	0.3	0	0	0	0	0	1.2	0.3
Spinach	0	99.9	0.1	0	0	0	0	75.2	0.1
Cleavers	0	0.4	99.5	0.1	0	0.1	0	7.5	1.5
Black bindweed	0	0.3	0	99.7	0	0	0	10.1	0.4
Radish	0	1.8	0.5	0	97.6	0	0.1	2.6	2.5
Rapeseed	0	0.5	0.9	0	0.4	97.9	0.4	2.3	2.1
Hemp-nettle	0	0.5	0.2	0	0.2	0.9	98.2	0.8	1.8
Total	1.2	75.3	7.5	10.1	2.5	2.2	0.8	57115	
Error	0	0.2	1.6	0.3	0.7	1.2	3.2		0.4

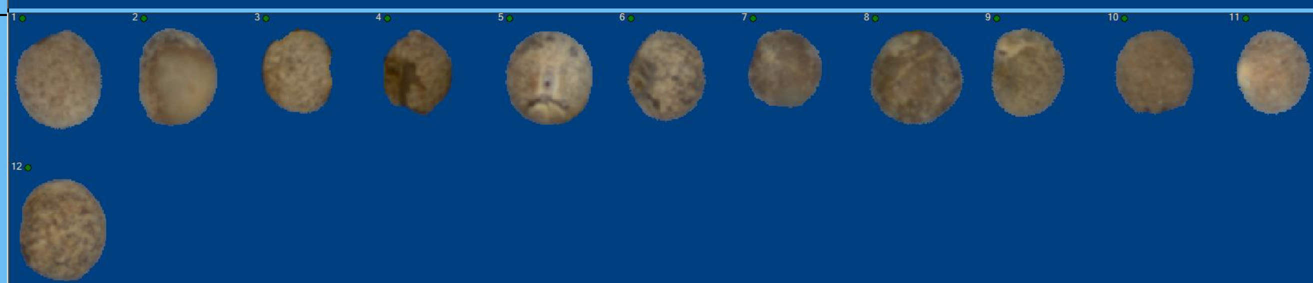
Classification Spinach vs Cleavers

Class	Example Images
Spinach classified as Spinach	
Spinach classified as Cleavers	

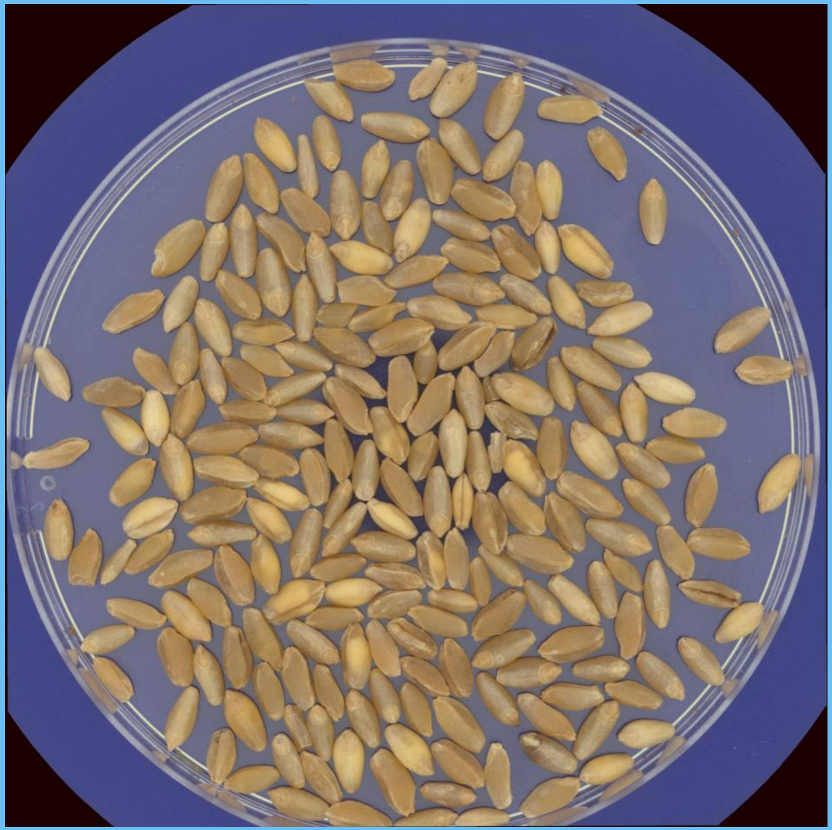
Classification Spinach vs Cleavers

Cleavers classified as cleavers

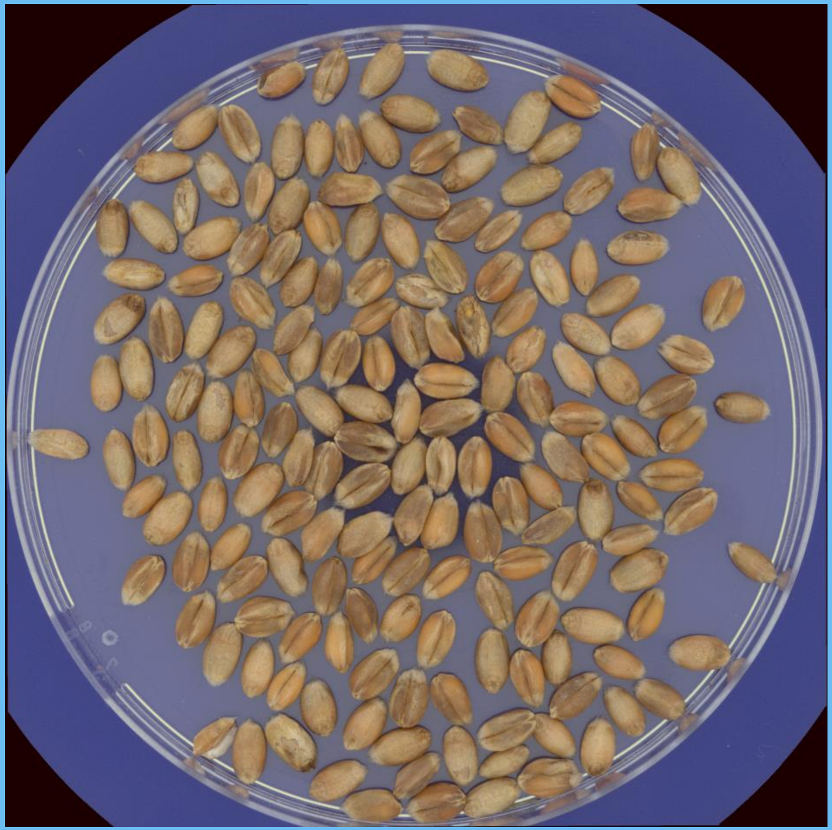
Cleavers classified as spinach



Pure samples 1 and 2 in sRGB

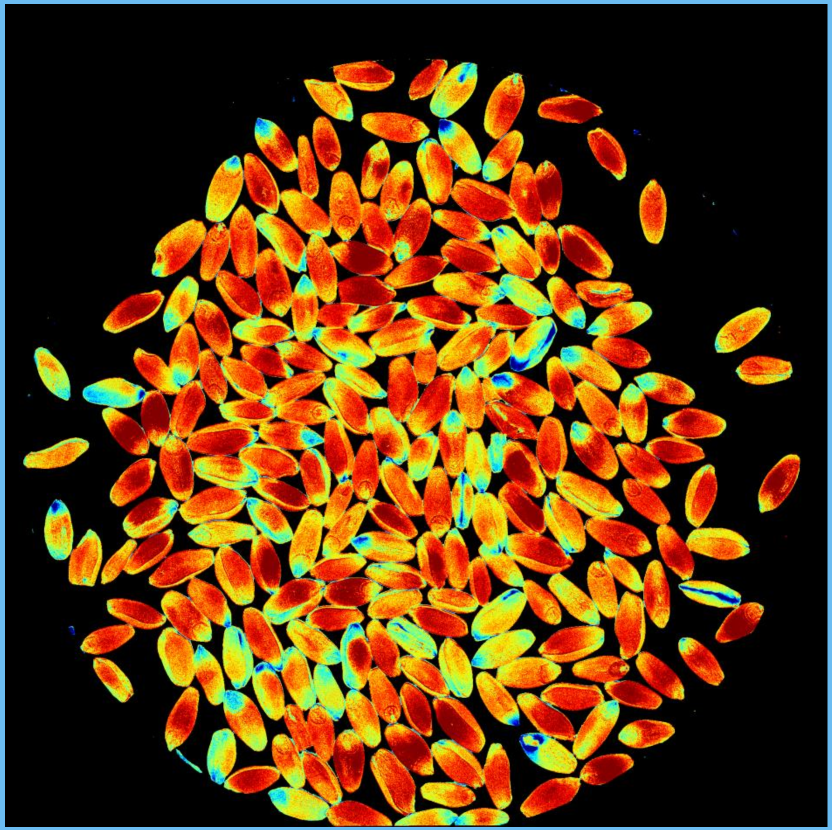


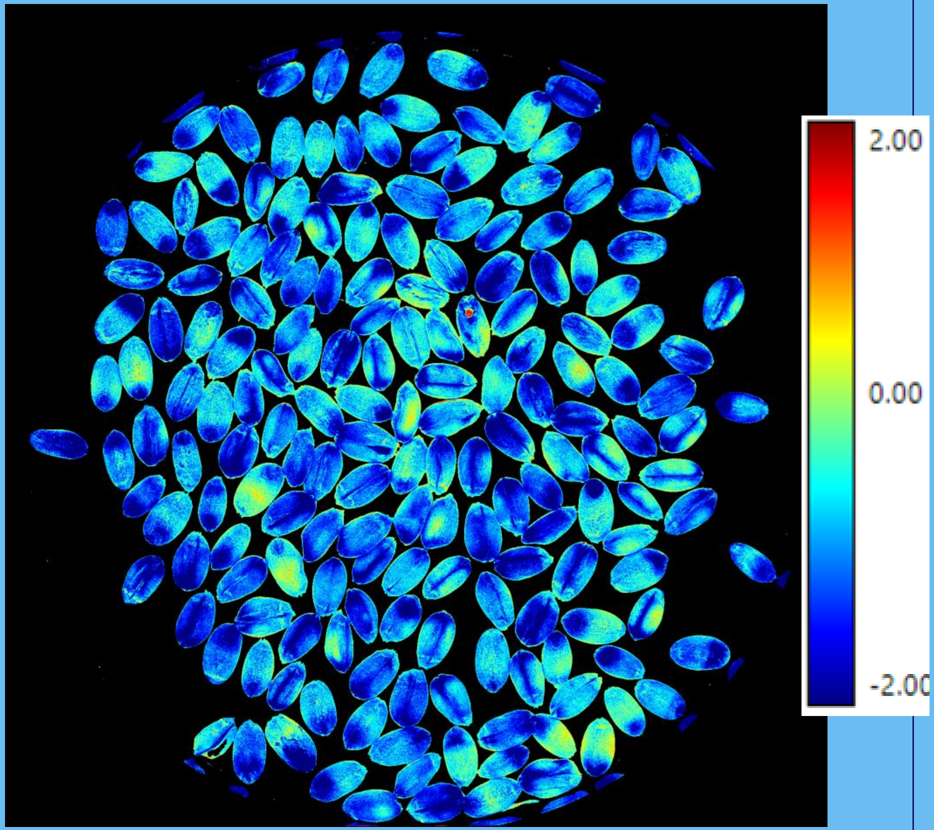
Durum



Common wheat

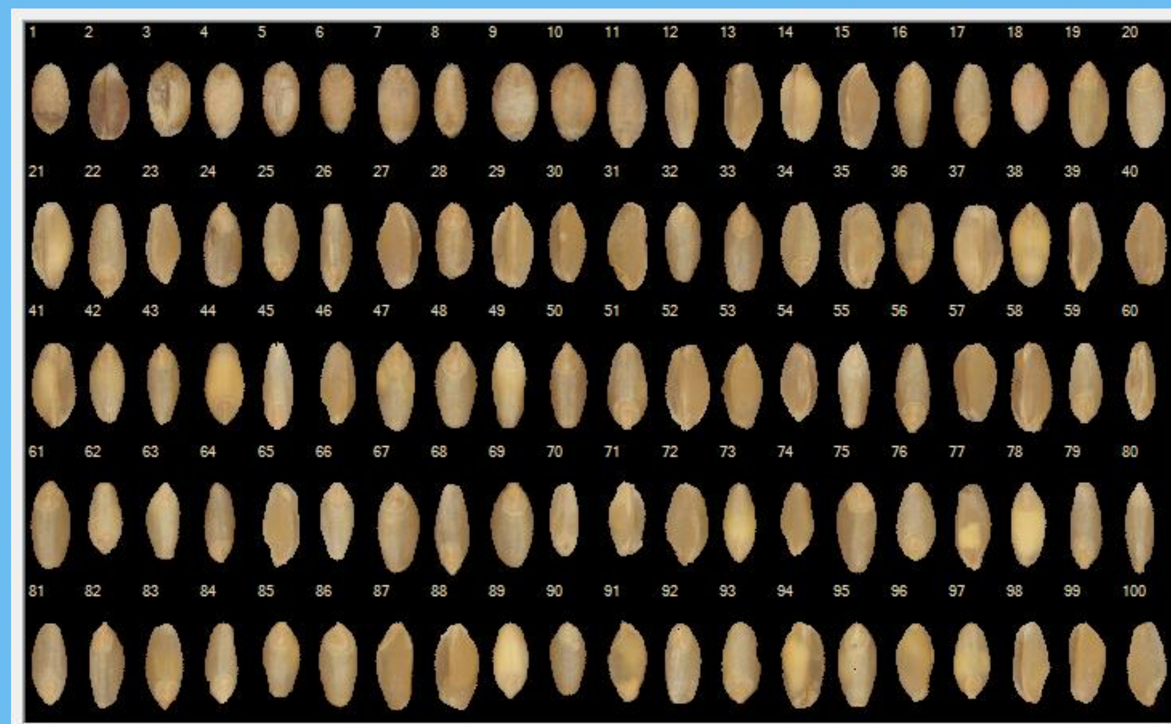
Pure samples 1 and 2 after nCDA





Sample 3

All 100 seeds automatically segmented and sorted according to likelihood of being durum



Result sheet from spectral info only

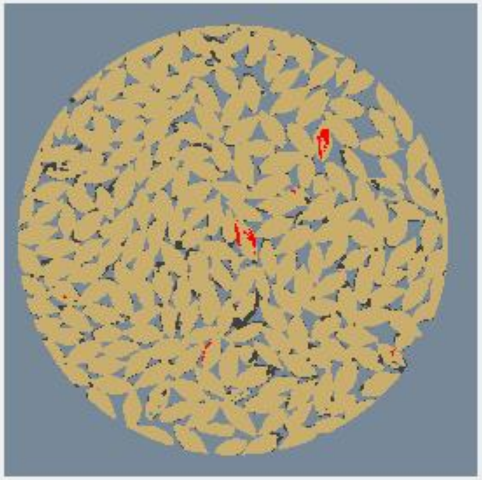
Sample number	Sample name	REAL # of Durum wheat seeds	EST # of Durum wheat seeds	REAL # of Aestivum wheat seeds	EST # of Aestivum wheat seeds	Total number of seeds
1	100% Durum control	300	300	0	0	300
2	100% Aestivum control	0	0	300	300	300
3	10% adulterated	90	89	10	11	100
4	3% adulterated	97	95	3	5	100
5	100% adulterated	0	0	100	100	100
6	3% adulterated	97	95	3	5	100
7	2% adulterated	98	98	2	3	100
8	5% adulterated	95	94	5	6	100
9	10% adulterated	90	90	10	11	100
10	0.5% adulterated	199	195	1	4	200
11	0% adulterated	100	98	0	2	100
12	0.5% adulterated	199	198	1	2	200
13	5% adulterated	95	95	5	5	100
14	5% adulterated	95	95	5	5	100
15	2% adulterated	88	87	2	3	90
16	10% adulterated	90	91	10	9	100
17	0% adulterated	100	99	0	1	100
18	100% adulterated	0	0	100	100	100
19	0.5% adulterated	199	197	1	0	200
20	2% adulterated	98	98	2	2	100
21	100% adulterated	0	0	100	100	100
22	0% adulterated	100	100	0	0	100
23	3% adulterated	97	96	3	4	100

SEED HEALTH

Seed health -Red Fusarium Grey molds

Recipe: Red Fusarium grey moulds ver 3-2

Plan: No Plan



Sample ID	Red Fusarium	Red Fusarium	Red Fusarium	Red Fusarium	Red Fusarium
EF01 Prestice 3_Capture1	2.674436	0	2.44979525	2	0
EF01 Prestice 3_Capture2	0.222490549	1.44846332	0	0	1
EF01 Prestice ny_Capture1	0.482127368	0	0.3215661	1	0
EF01 Prestice ny_Capture2	3.59720349	2.03966546	3.03879118	4	2

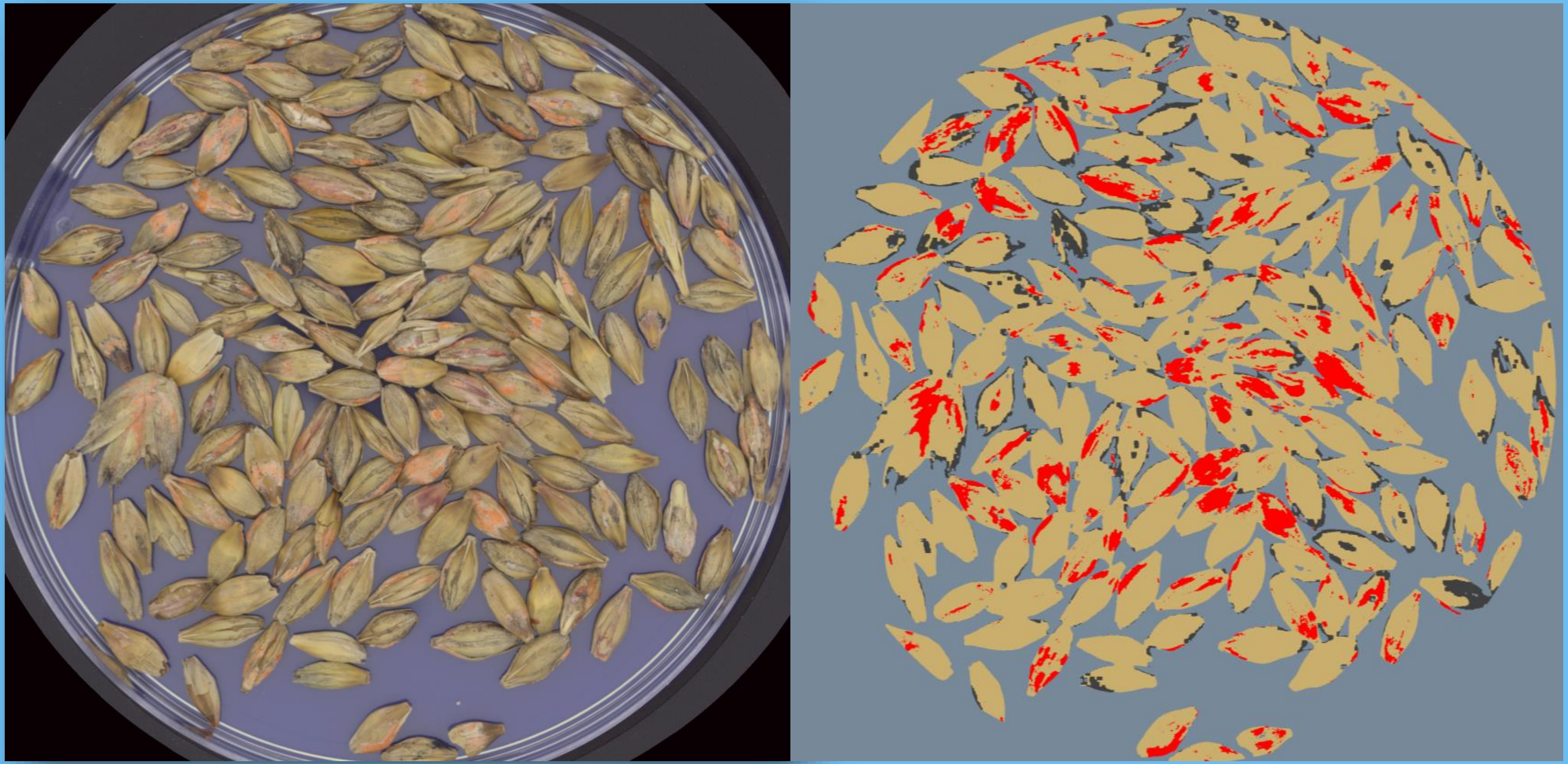
Next Sample Data

Sample ID: Sample Note:

Auto Number: 00001 Filename: Red Fusarium grey moulds ver 3-2__Capture1

Capture Number: 1 of 2 *SessionName_SampleID_CaptureNumber*

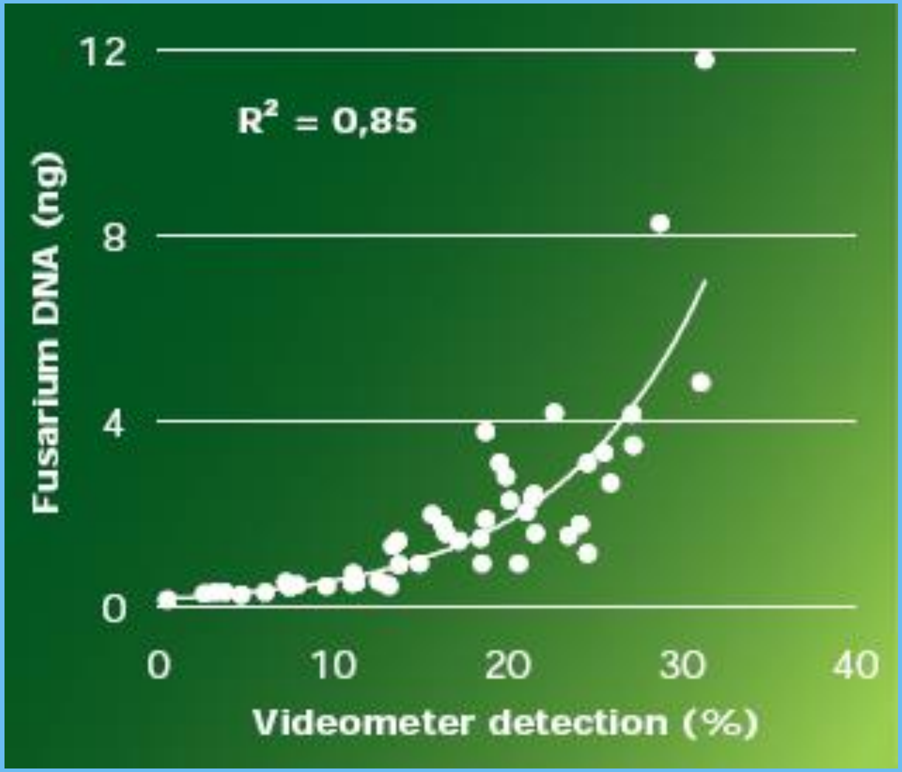
Result image



The red color = red, orange or purple area on kernels
The gray color = grey and black moulds
Brownish = Barley without moulds

Validation by Carlsberg Research Center

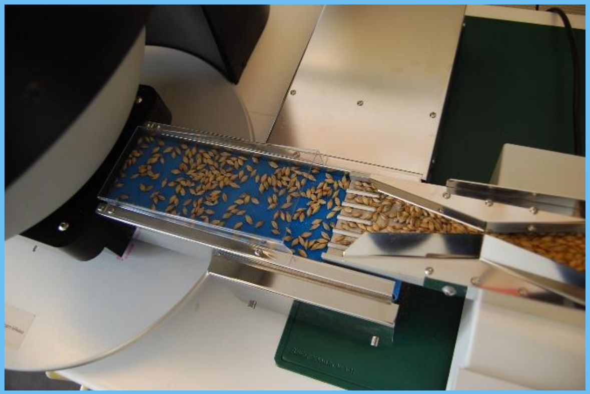
Comparison between **VideometerLab®** measurements and the level of *Fusarium* DNA quantified by **qPCR**

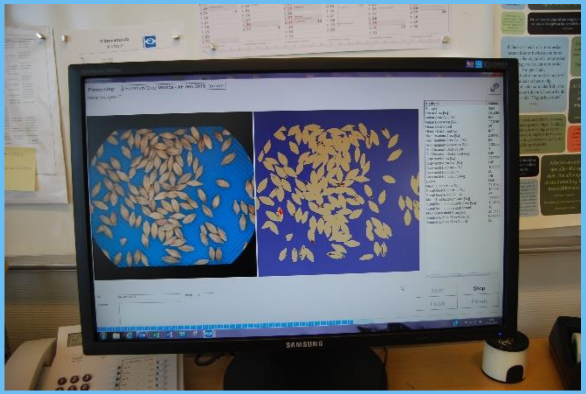


→ **Excellent correlation with Fusarium DNA level (R^2=0,85)**

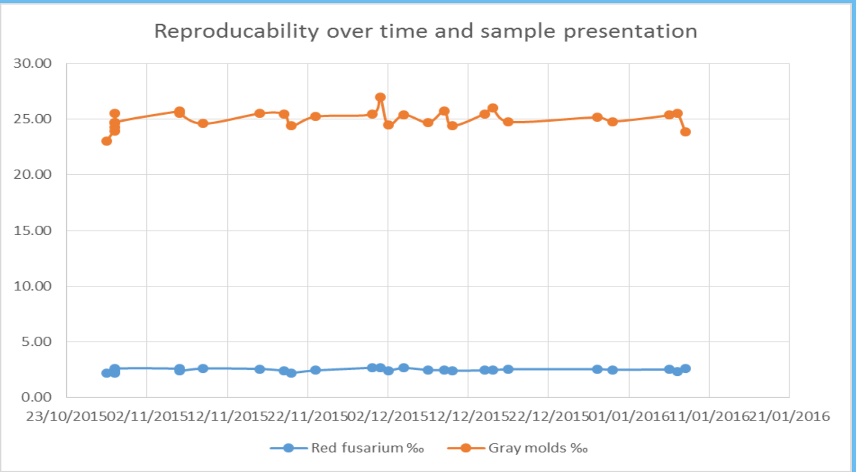
Inspection of larger samples

Analysis sequence

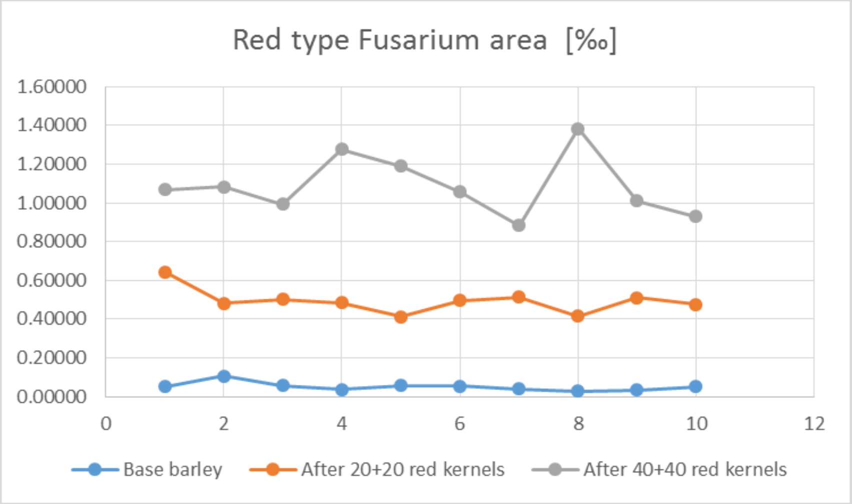


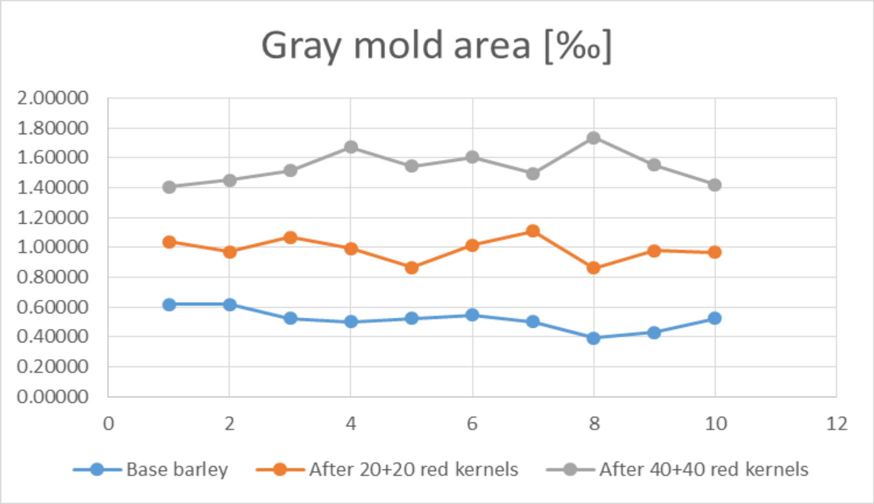


Results



560 g sample



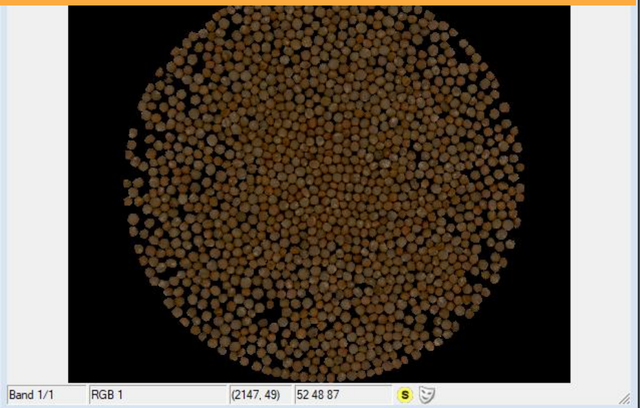


High sensitivity and reproducibility

SEED GERMINATION AND VIGOR

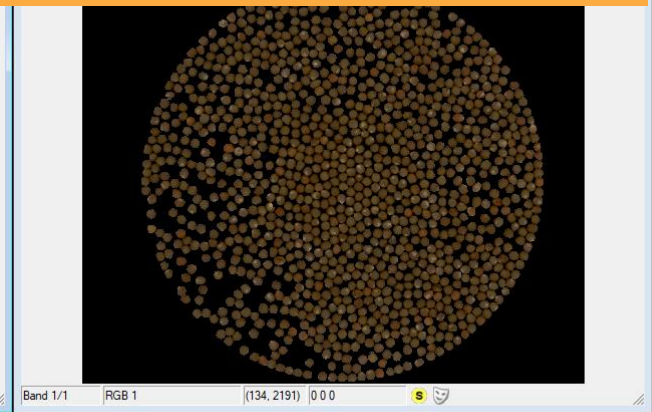
Hybrid Cabbage (sRGB)

Germination 87%

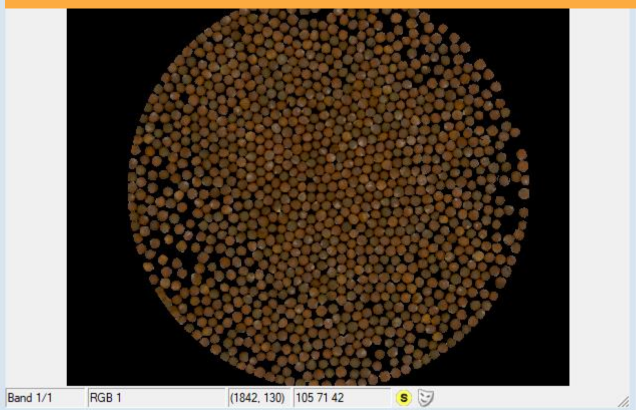


Germination 92%

Germination 84%



Germination 54%

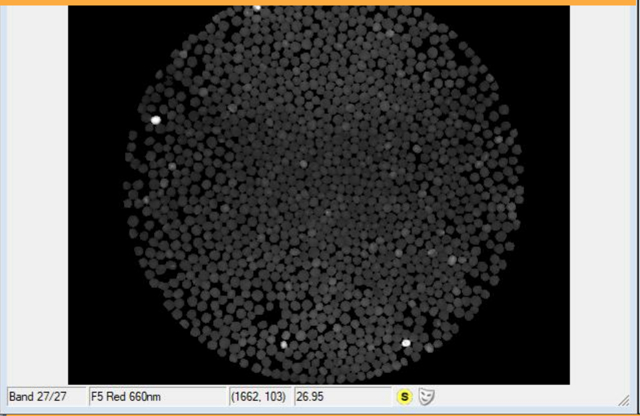


Germination 73%

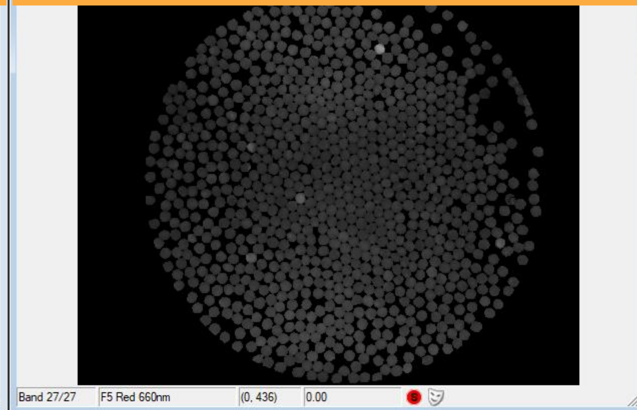
Germination 47%

Hybrid cabbage red fluorescence

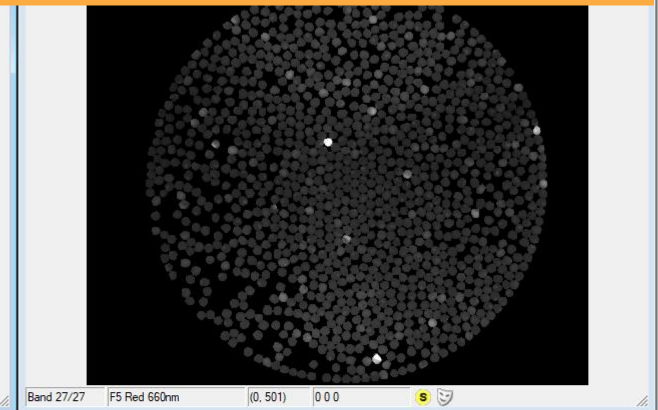
Germination 87%



Germination 92%

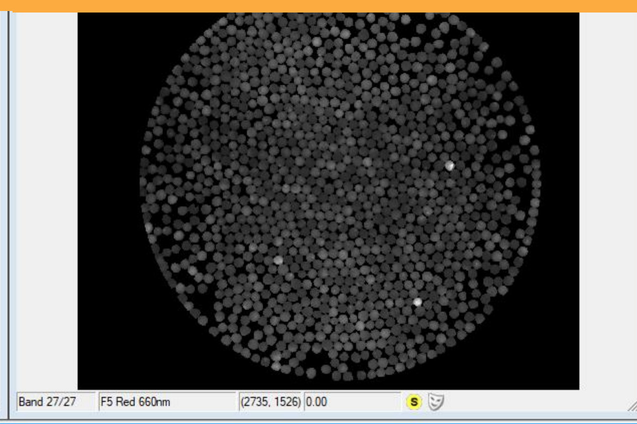


Germination 84%

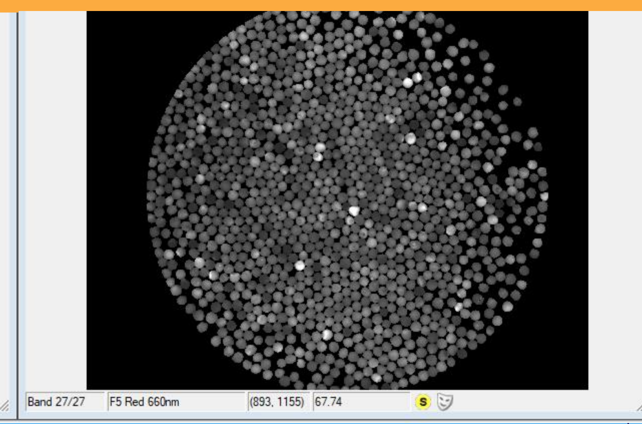


Germination 54%

Germination 73%



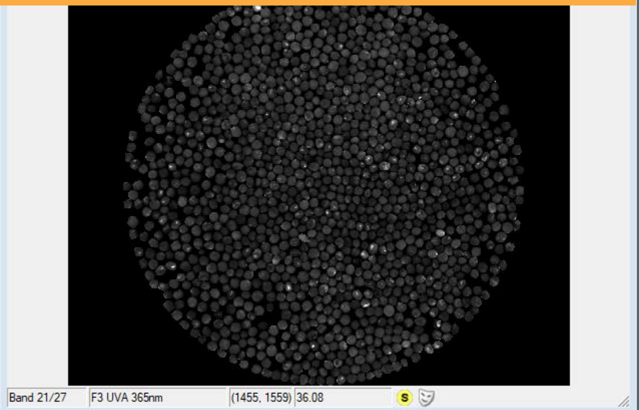
Germination 47%



(660 nm, >700 nm)

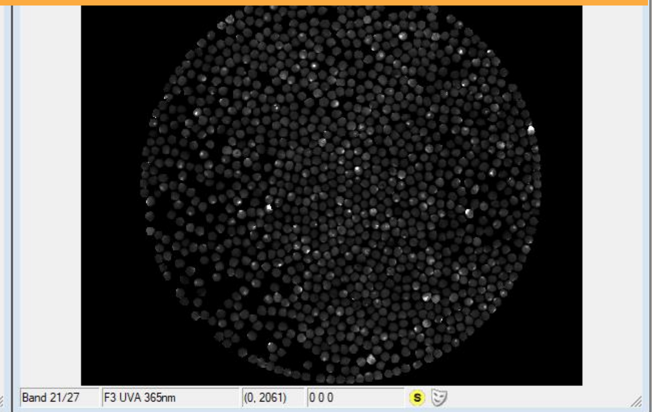
Hybrid cabbage UV fluorescence

Germination 87%

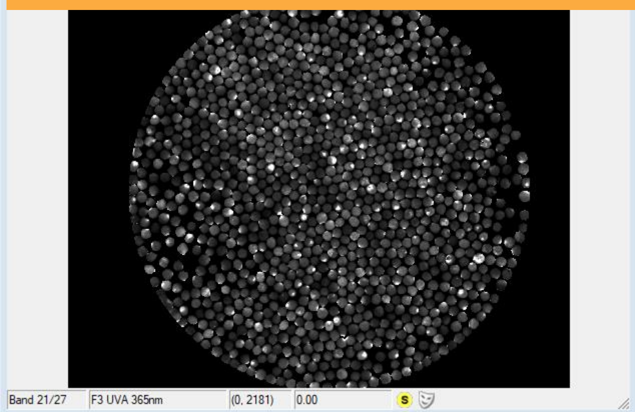


Germination 92%

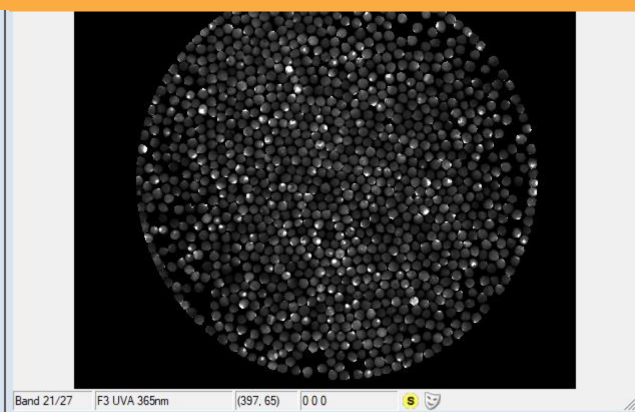
Germination 84%



Germination 54%



Germination 73%

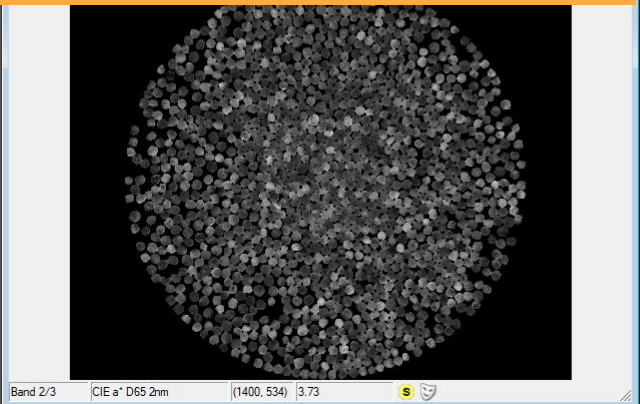


Germination 47%

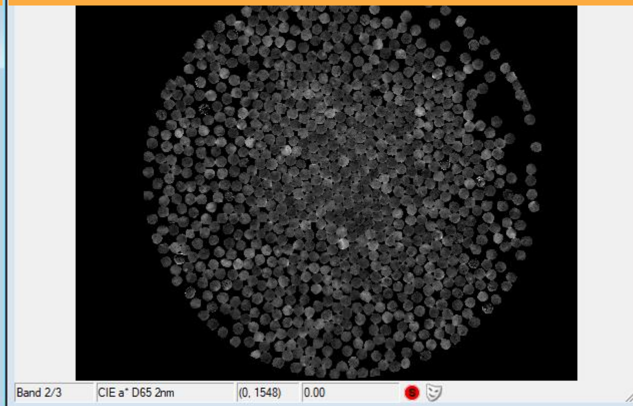
(365 nm, >500 nm)

Hybrid cabbage greeniness

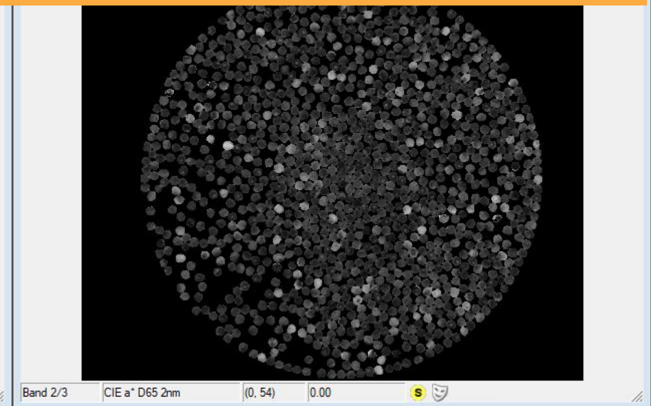
Germination 87%



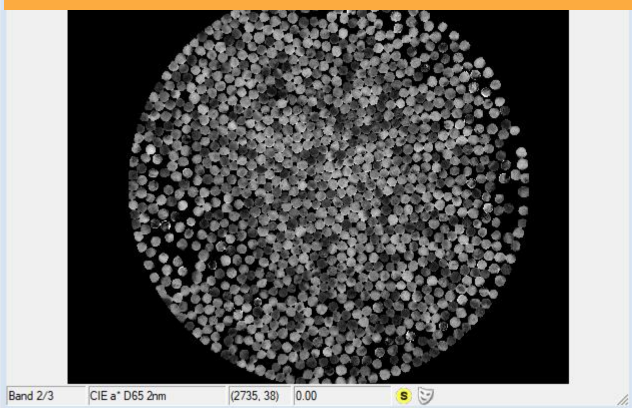
Germination 92%



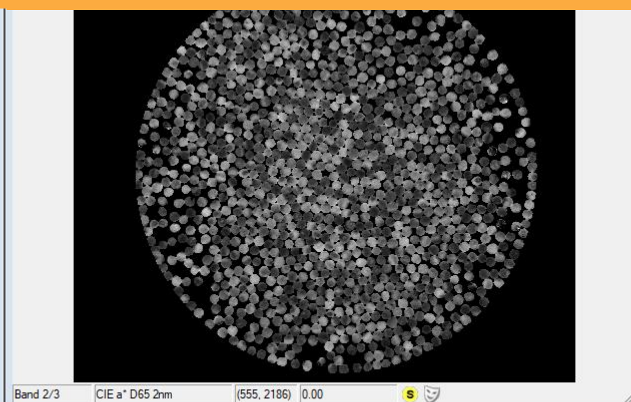
Germination 84%



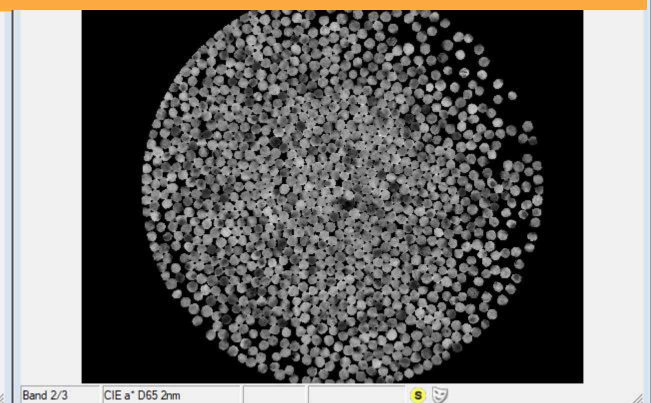
Germination 54%



Germination 73%

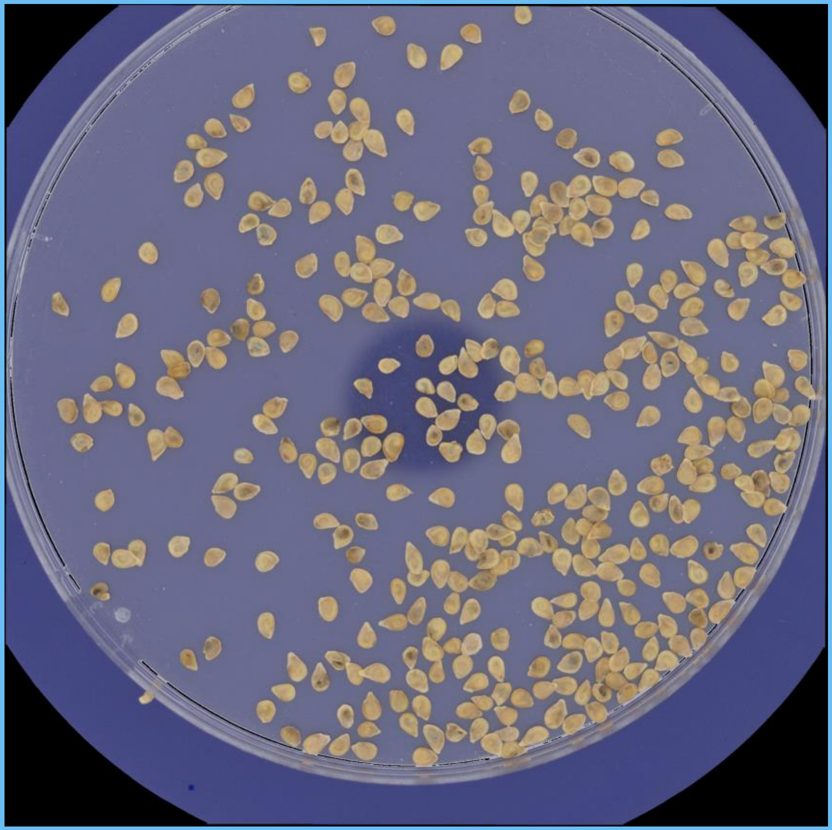


Germination 47%

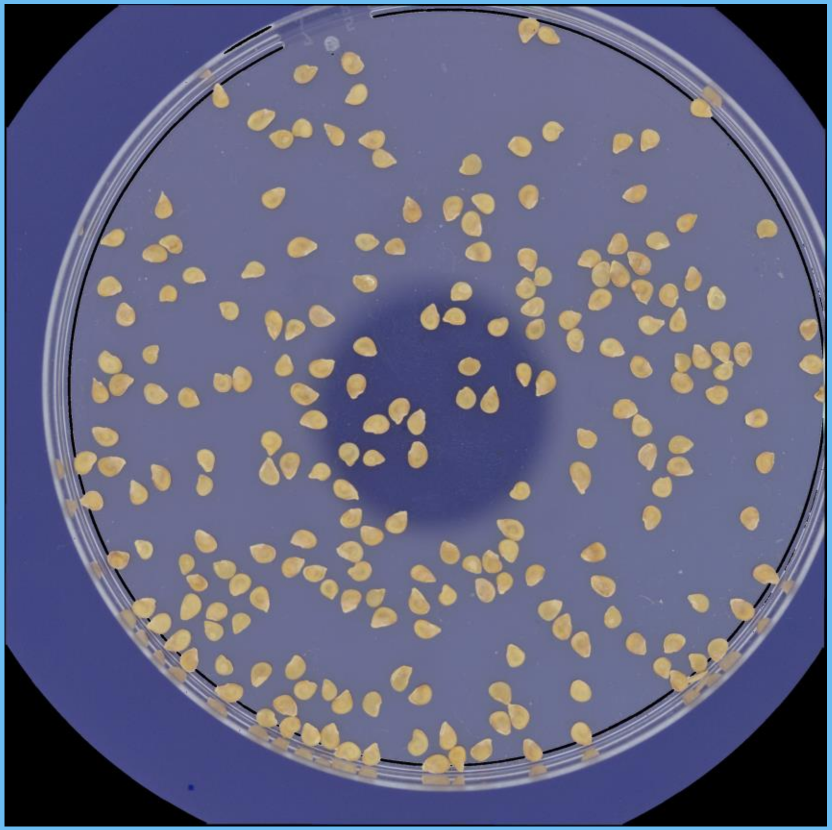


Tomato seed viewed in sRGB (D65)

Lot 1 (low chlorophyll)

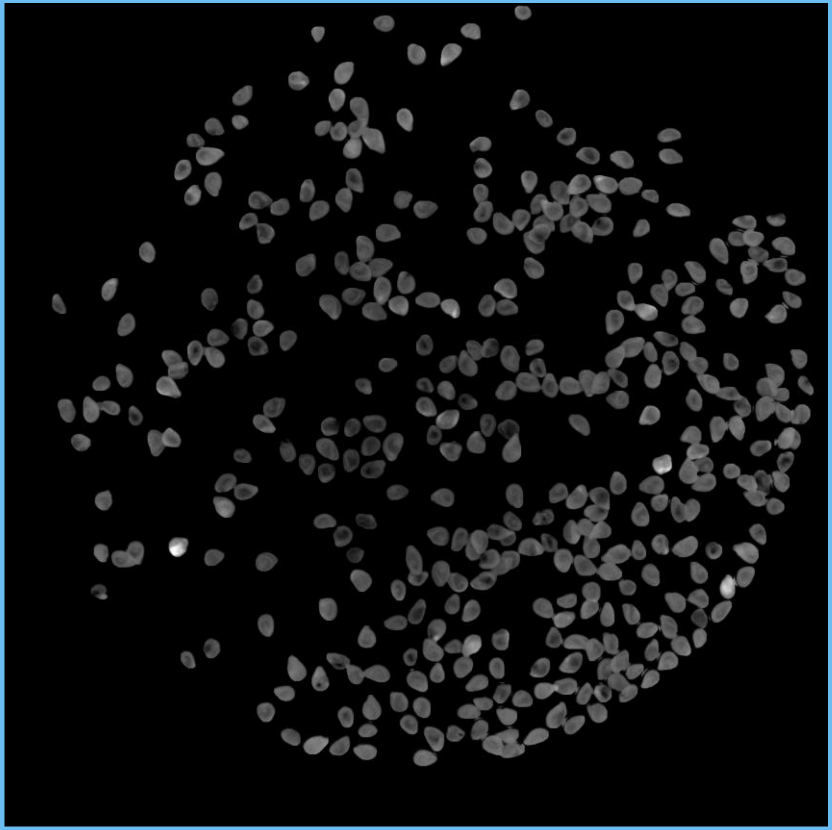


Lot 2 (high chlorophyll)

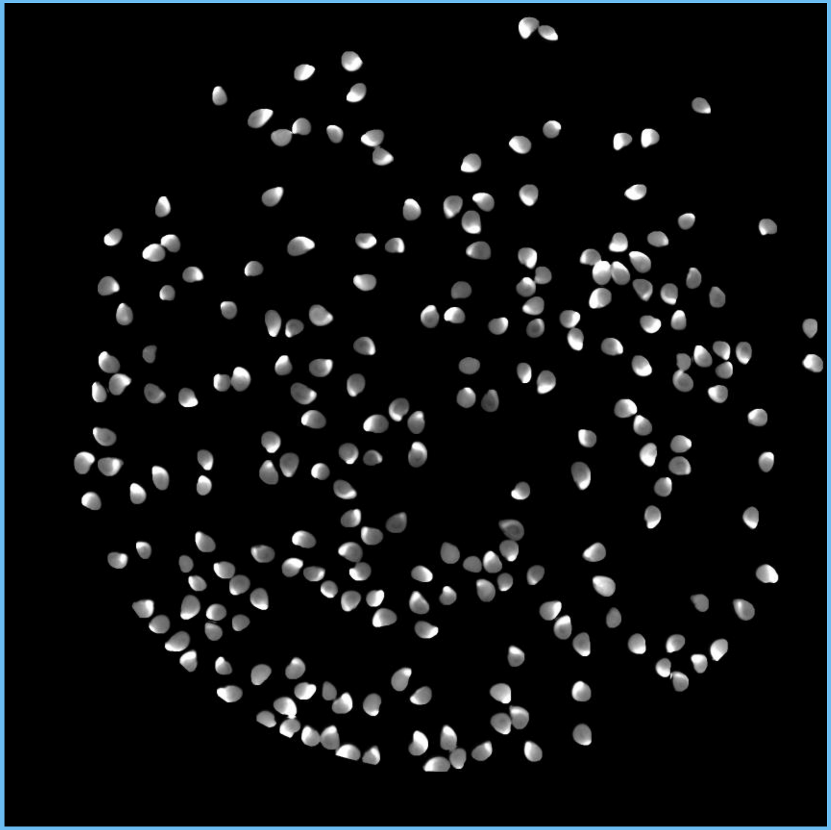


Tomato seed chlorophyll A fluorescence

Lot 1 (low chlorophyll)

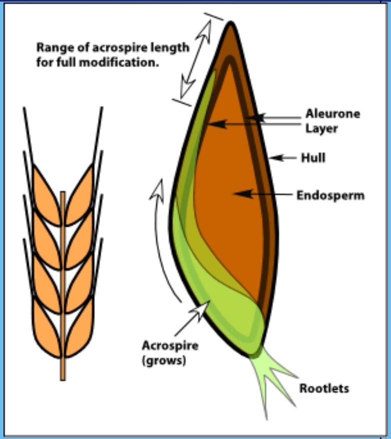


Lot 2 (high chlorophyll)



Germination - Acrospire length

The germination process can be followed by measuring the length of the acrospires inside the kernel.



- Boil the germinated malt 10 minutes and leave the kernels in the water for a ½ hour.
- Place 26 kernels in the presentation tray – with the front side up.
- Insert the sample holder in the VideometerLab.
- Activate measurement
- The Acrospire model measures the length of the Acrospire and compare it with the length of each seed.

Result within 20 seconds.

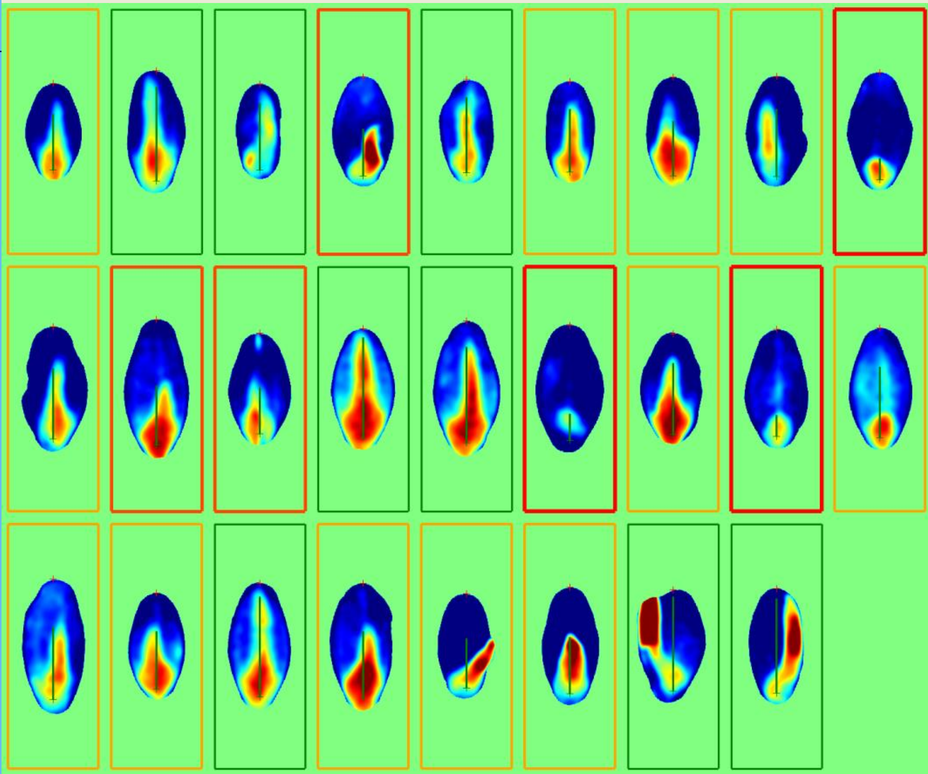
A fast and accurate way to calculate the mean acrospire length - to know when to start the killing process!

Acrospire length - User interface

Session: New Acrospire length bm 2003

Recipe: New Acrospire length bm 2

Plan: No Plan



Sample ID	Note	#Total	Mean Relativ	#Group1	#Group2	#Group3	#Group4	#Gro
Prøve0_1		25	0,575	2	5	15	3	0
Prøve0_2		26	0,5576923	0	10	13	3	0
Prøve0_3		25	0,585	0	9	11	5	0
Prøve0_4		26	0,6298077	0	7	13	5	1
Prøve1_1		26	0,677884638	1	6	6	13	0
Prøve1_2		26	0,7692308	0	0	11	15	0
Prøve1_3		26	0,7355769	0	2	12	11	1
Prøve2_0		26	0,7019231	0	1	16	9	0
Prøve2_1		26	0,6923077	0	1	17	8	0
Prøve2_2		26	0,721153855	0	3	10	13	0
Prøve2_3		26	0,711538434	0	2	13	11	0
Prøve2_4		26	0,7355769	0	2	12	11	1

Resume

Finish Sessio

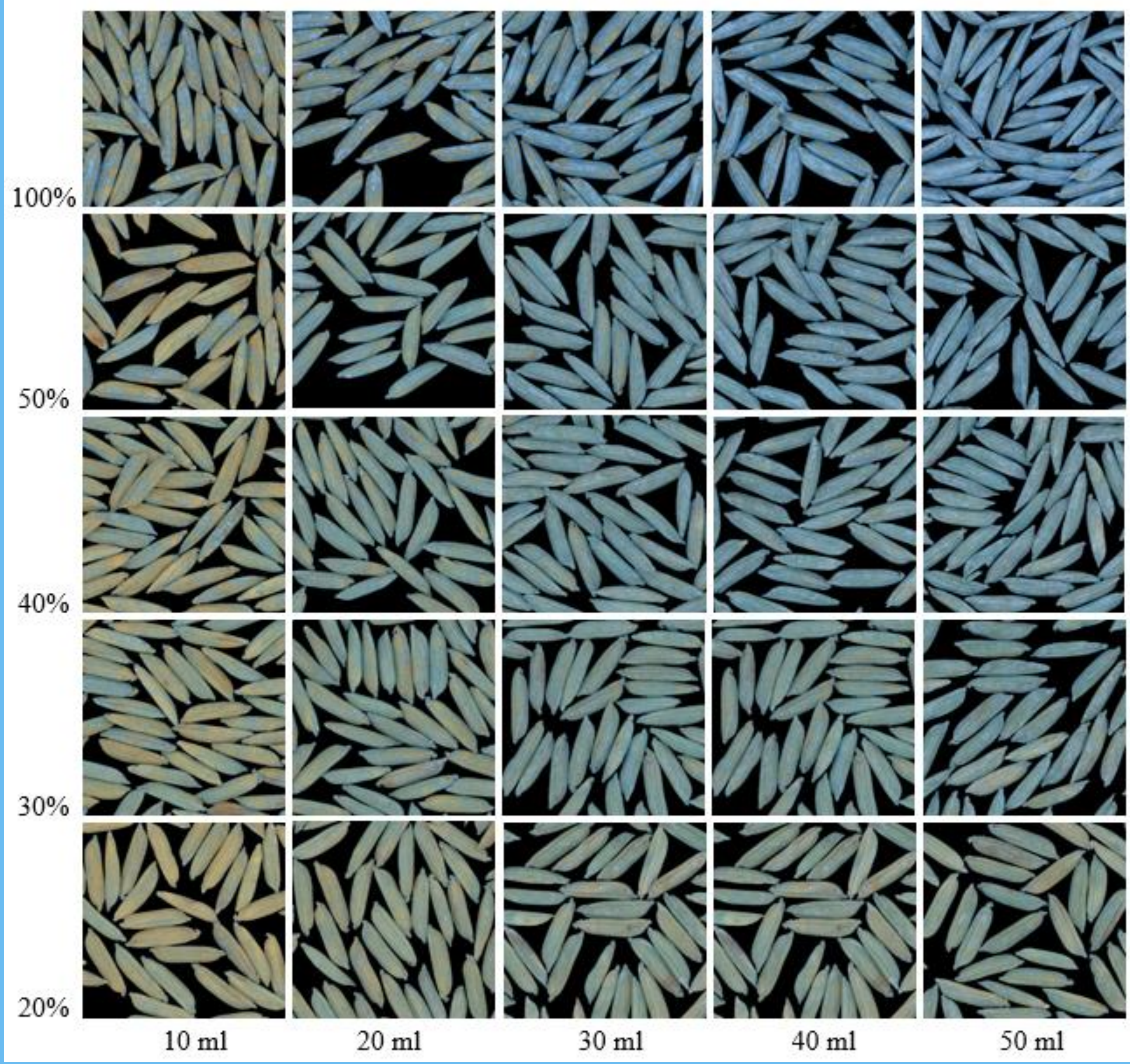
SEED COATING

Seed coating analysis

	10 ml	20 ml	30 ml	40 ml	50 ml
100%	10	20	30	40	50
50%	5	10	15	20	25
40%	4	8	12	16	20
30%	3	6	9	12	15
20%	2	4	6	8	10

Amount of polymer applied as a function of:

1. Concentration of polymer
2. Volume of coating liquid



Coating array

Easy coating model generation

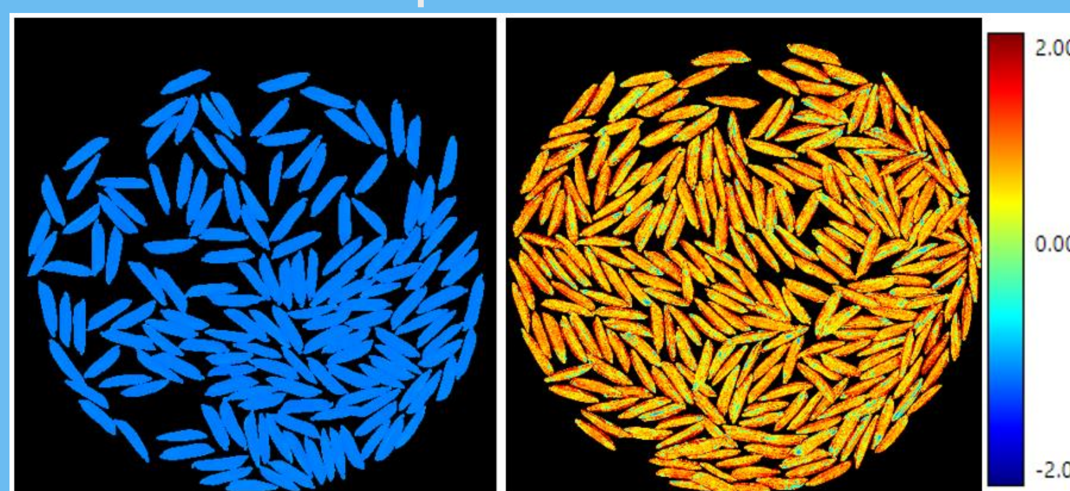
Input

Control image
(non-coated seeds)

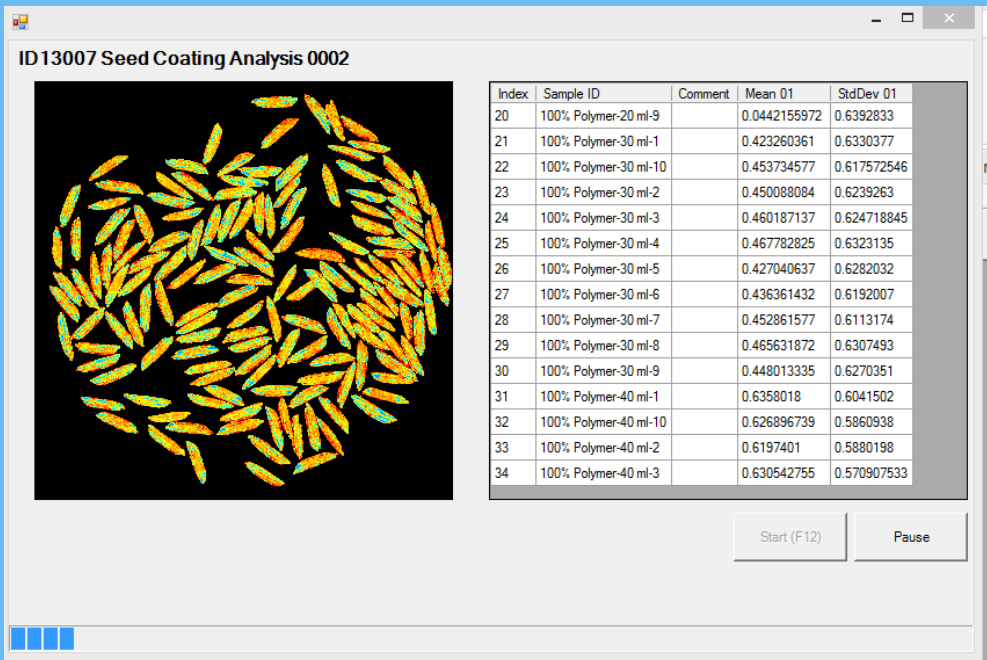
Ref. image
(coated seeds)

Output model

Loading map
(non-coated seeds)



Seed coating analysis



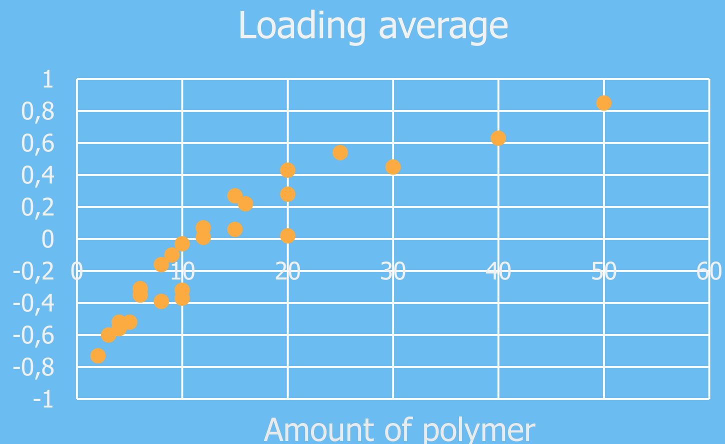
Index	Sample ID	Comment	Mean 01	StdDev 01
20	100% Polymer-20 ml-9		0.0442155972	0.6392833
21	100% Polymer-30 ml-1		0.423260361	0.6330377
22	100% Polymer-30 ml-10		0.453734577	0.617572546
23	100% Polymer-30 ml-2		0.450088084	0.6239263
24	100% Polymer-30 ml-3		0.460187137	0.624718845
25	100% Polymer-30 ml-4		0.467782825	0.6323135
26	100% Polymer-30 ml-5		0.427040637	0.6282032
27	100% Polymer-30 ml-6		0.436361432	0.6192007
28	100% Polymer-30 ml-7		0.452861577	0.6113174
29	100% Polymer-30 ml-8		0.465631872	0.6307493
30	100% Polymer-30 ml-9		0.448013335	0.6270351
31	100% Polymer-40 ml-1		0.6358018	0.6041502
32	100% Polymer-40 ml-10		0.626896739	0.5860938
33	100% Polymer-40 ml-2		0.6197401	0.5880198
34	100% Polymer-40 ml-3		0.630542755	0.570907533

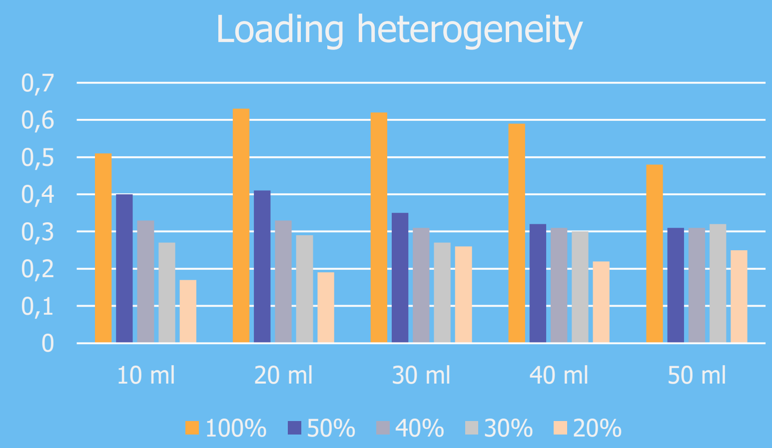
Loading average

	10 ml	20 ml	30 ml	40 ml	50 ml
100%	-0.37	0.02	0.45	0.63	0.85
50%	-0.52	-0.03	0.27	0.43	0.54
40%	-0.56	-0.16	0.07	0.22	0.28
30%	-0.60	-0.31	-0.10	0.01	0.06
20%	-0.73	-0.52	-0.35	-0.39	-0.32

Loading heterogeneity

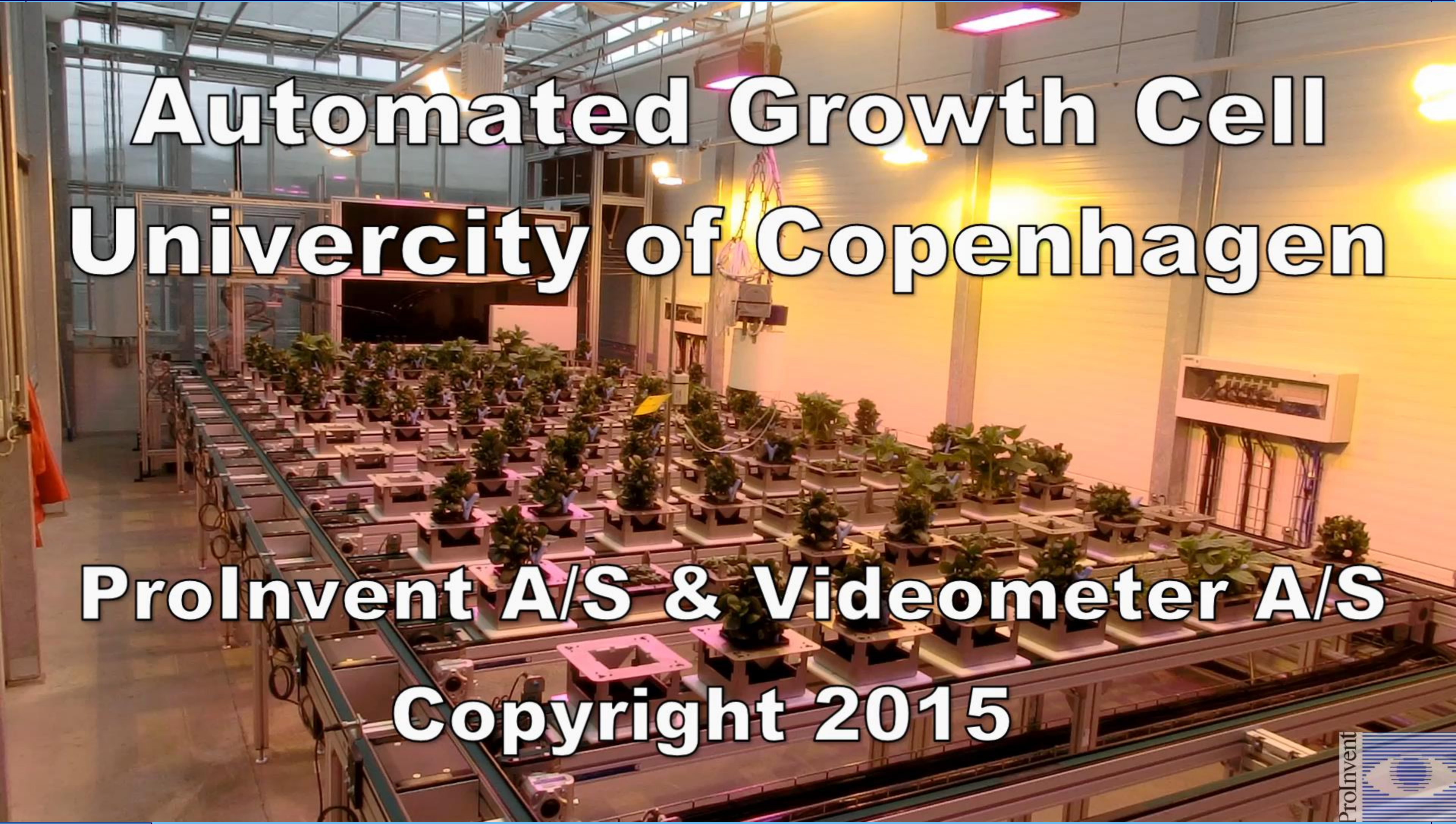
	10 ml	20 ml	30 ml	40 ml	50 ml
100%	0.51	0.63	0.62	0.59	0.48
50%	0.40	0.41	0.35	0.32	0.31
40%	0.33	0.33	0.31	0.31	0.31
30%	0.27	0.29	0.27	0.30	0.32
20%	0.17	0.19	0.26	0.22	0.25





OTHER VIDEOMETER PLANT IMAGING PLATFORMS

Greenhouse phenotyping - PhenoLab

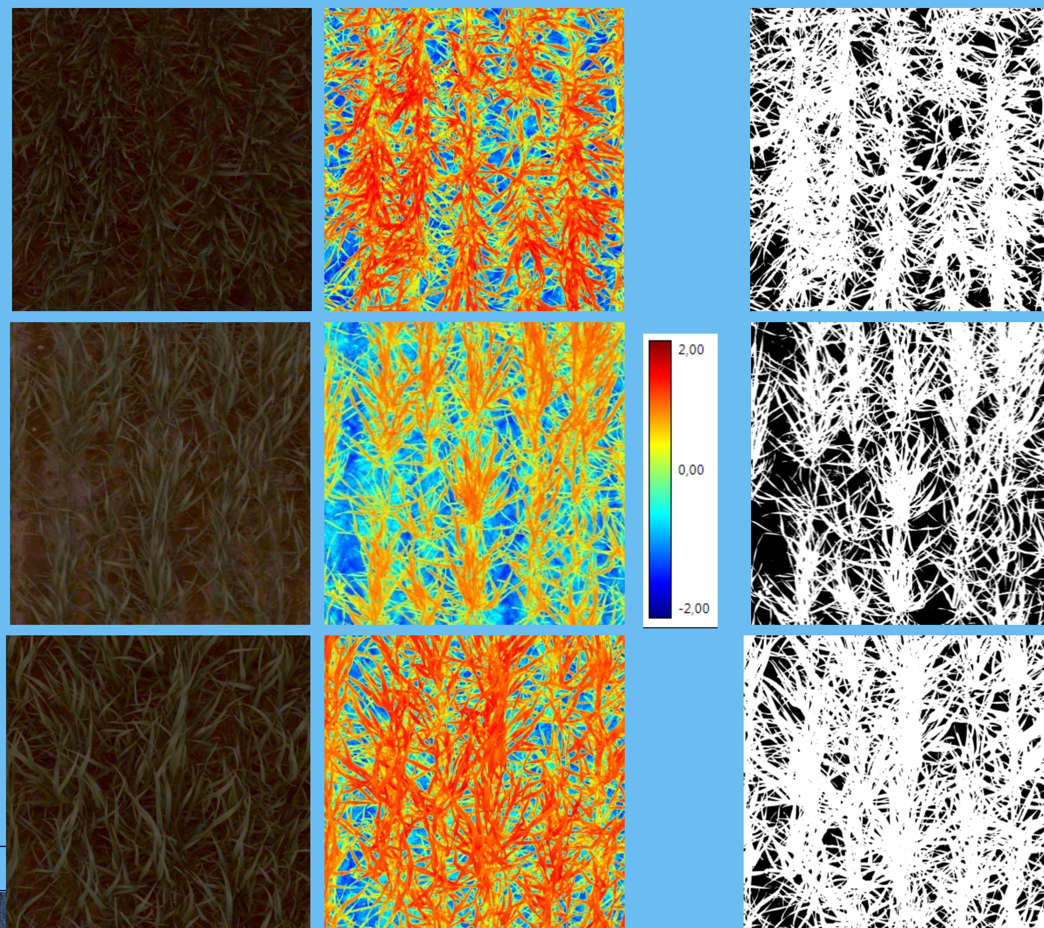


Field phenotyping - PhenoField

Photos: University of Copenhagen, Department of Plant and Environmental Sciences

Spring wheat

Sandy loam, nitrogen level 100 kg N/ha, planned for 350 plants/m2



Genotype - Spring wheat	Soil coverage (%)	Green index / Chlorofyl index (0-2)
1) Økilde	72,7	0,96
2) Quintus	56,9	0,89
3) KWS Scirocco	79,5	0,99

Source: Jesper Svensgaard, University of Copenhagen, Department of Plant and Environmental Sciences
jesv@life.ku.dk

RadiMax at University of Copenhagen

RadiMax
root deeper | produce more

DLF SEEDS & SCIENCE

Nordic Seed

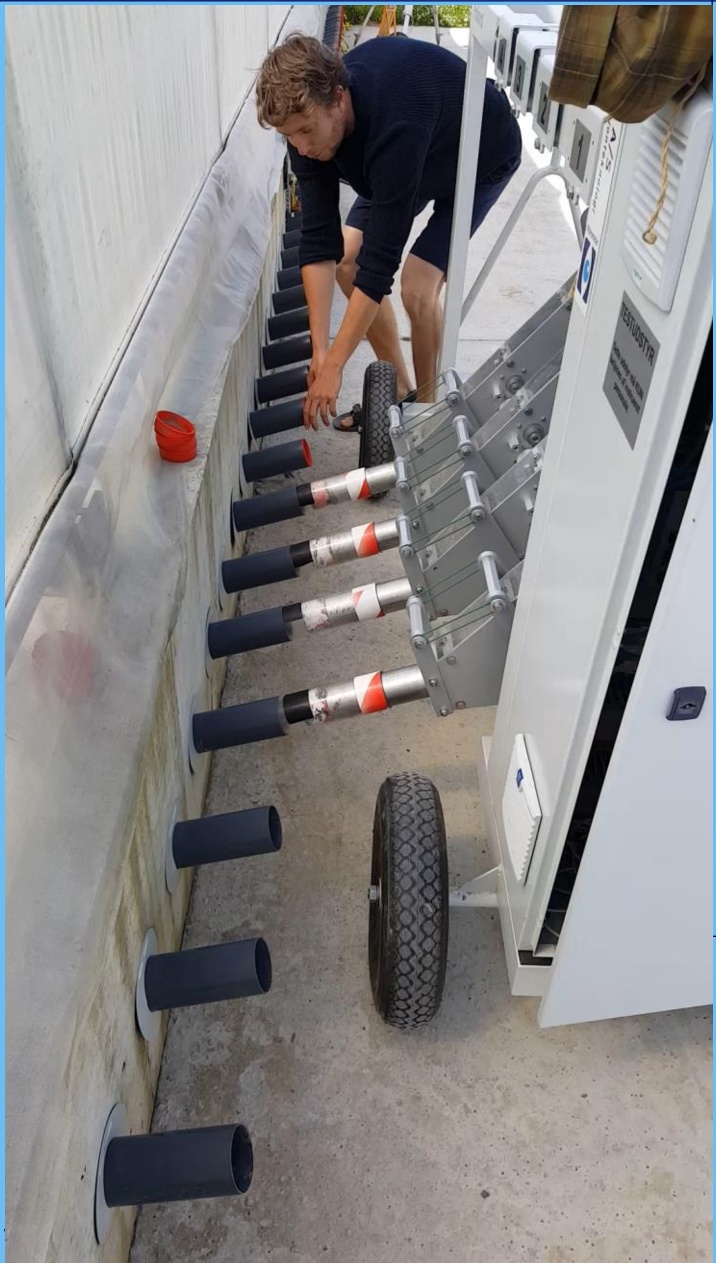
LKF Vandel

Sejet planteforædling

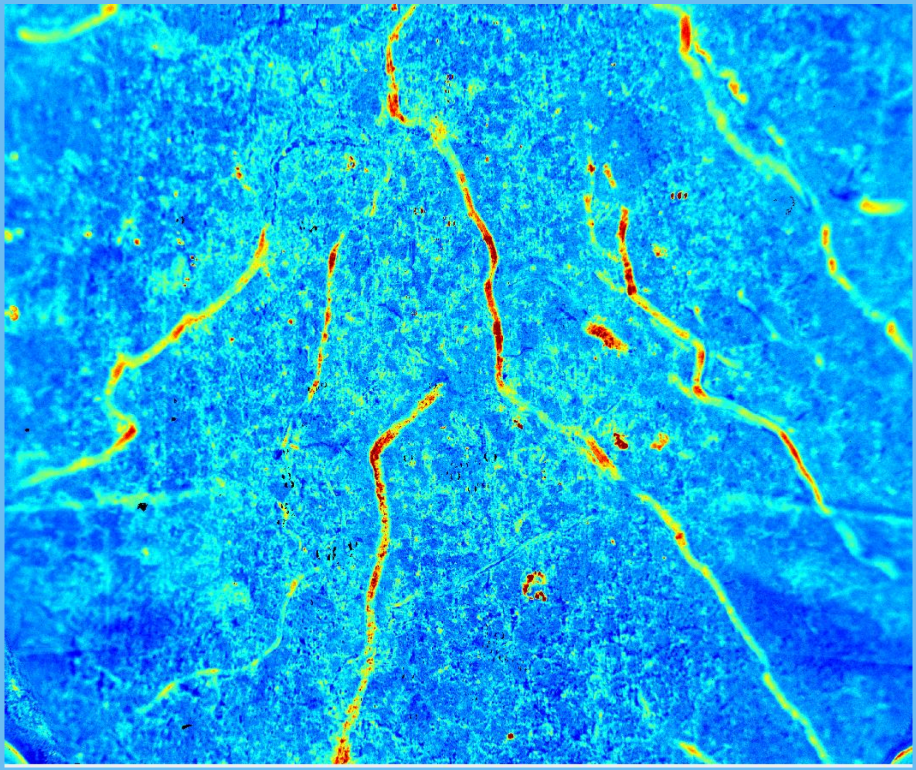
Innovation Fund Denmark
RESEARCH, TECHNOLOGY & GROWTH

CROP INNOVATION DENMARK
- from genes to seeds

Rhizosphere imaging



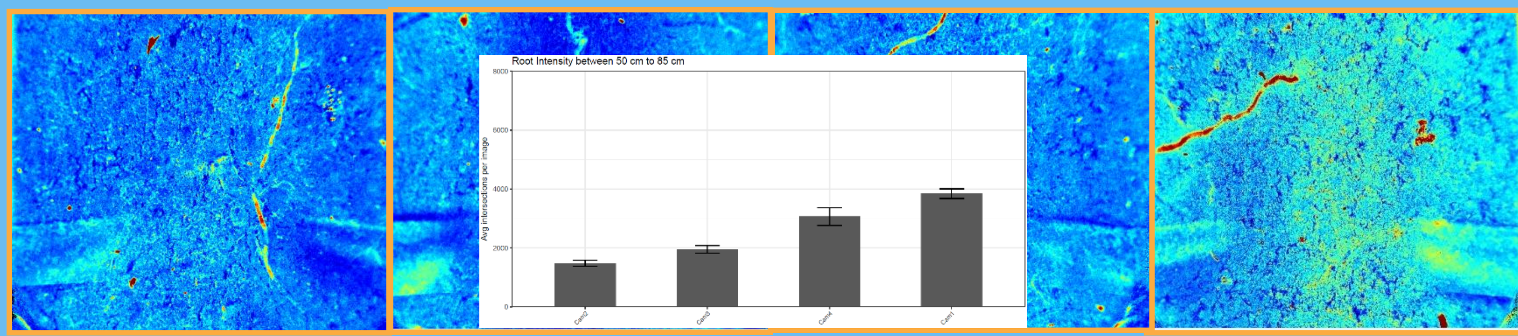
Spectral imaging detects live roots in all tested soils types



Slide source: KU

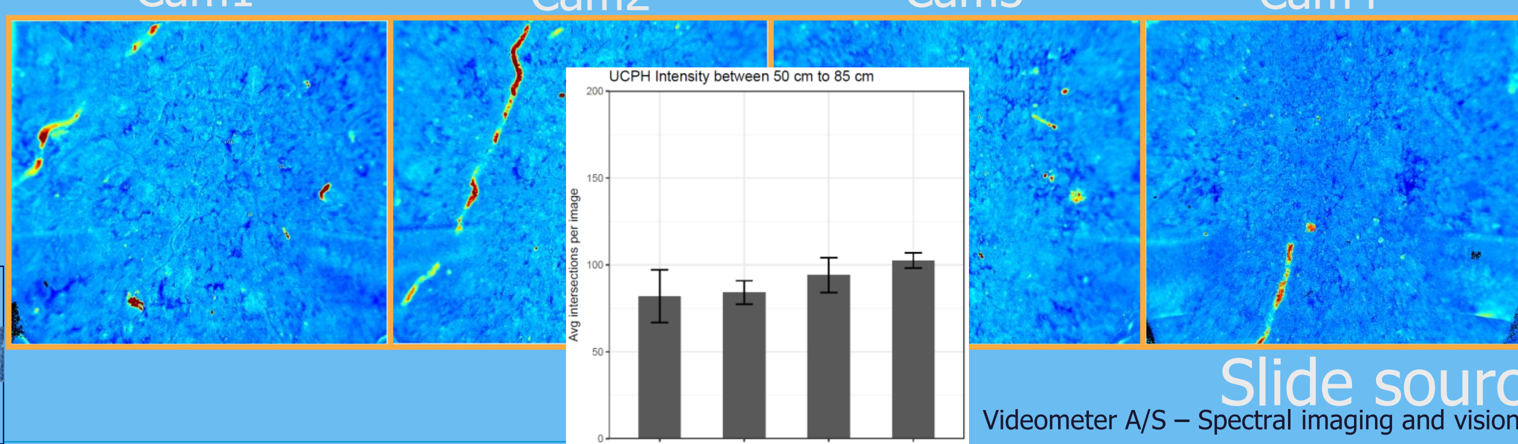
RadiMax calibration

Cam1 Cam2 Cam3 Cam4



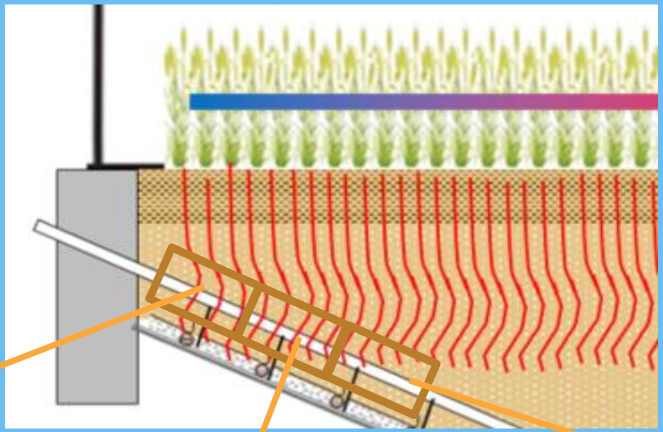
Light calibration step and new background adjustments step

Cam1 Cam2 Cam3 Cam4

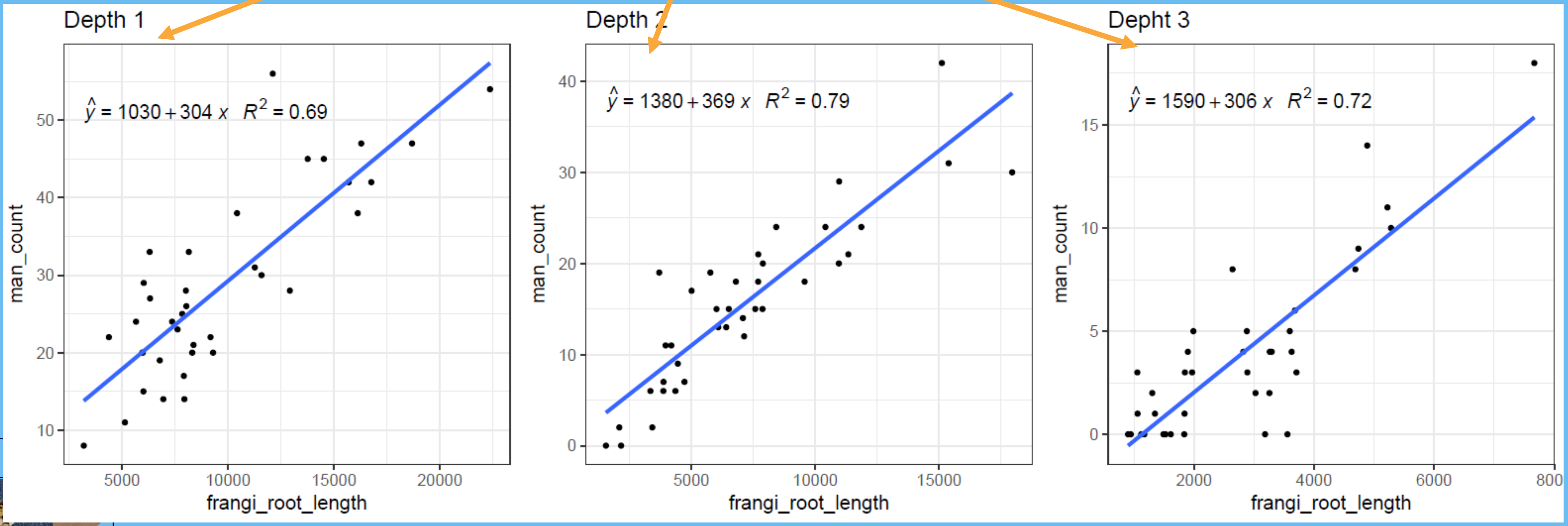


Slide source: KU

First validation experiment on RadiMax



Slide source: KU



Questions?