AARHUS UNIVERSITY

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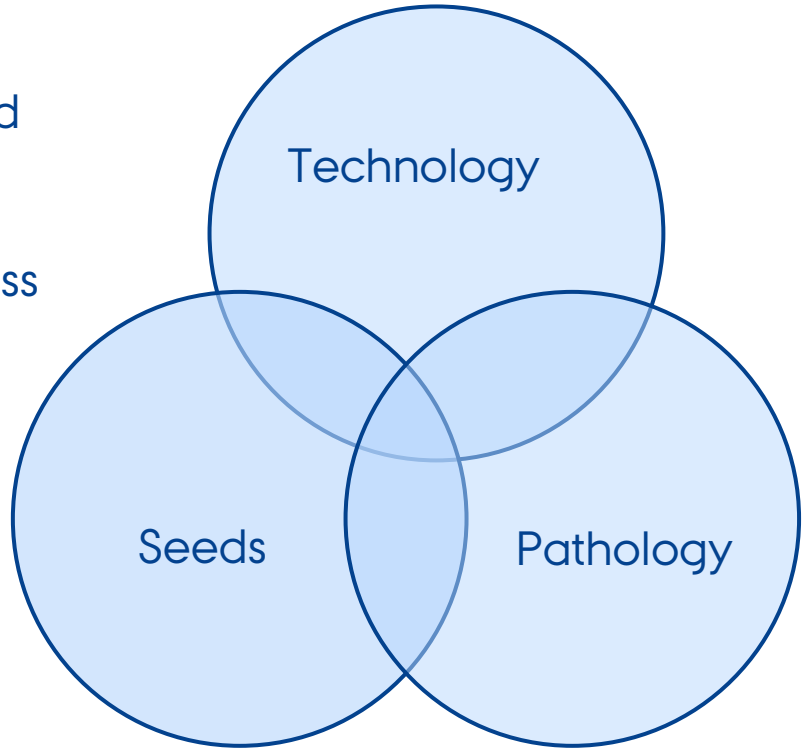
# Non-destructive techniques in seed quality determination

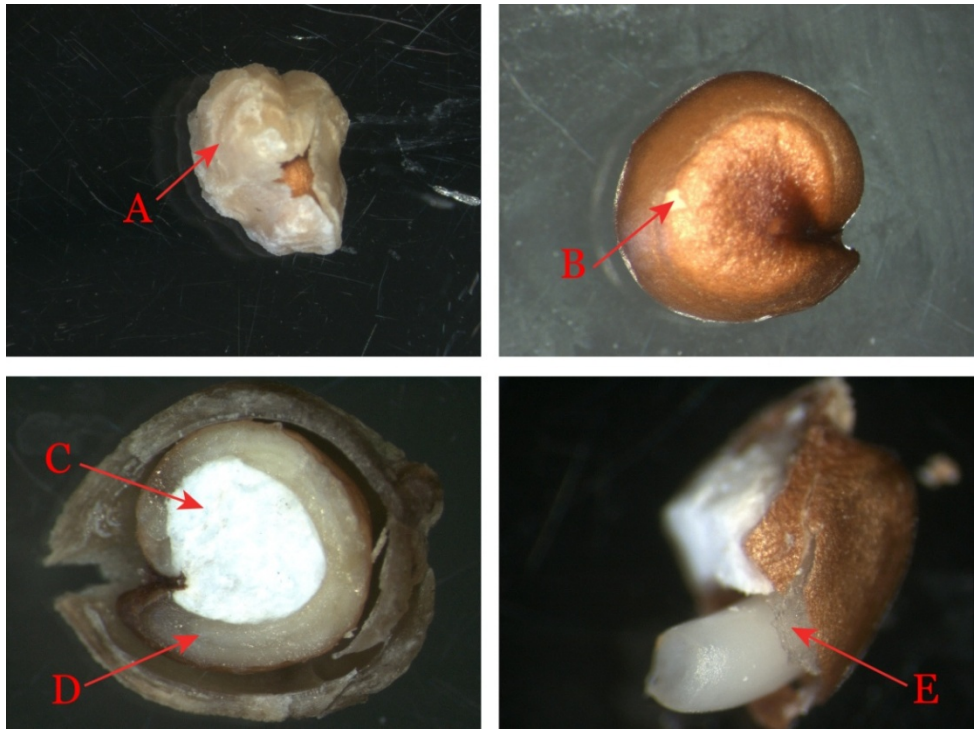
Merete Halkjær Olesen
Aarhus University
Science & Technology
Department of Agroecology

# Outline

- Seed anatomy and the importance of seed quality (germination and seed health)
- Non-destructive technologies used to assess various aspects of seed quality
- Results from PhD project



# Seed anatomy




Spinach seed. A: Seed with pericarp, B: True seed, brown testa, C: Perisperm, D: Embryo, E: Micropylar endosperm

# Germination

Any initial variation in plant size will increase as the crop matures, the larger plants in the population will continue to secure proportionally more of the resources available.

Komatzuna -Mizuna -Pak Choi -Chinese cabbage -Spinach

# Seed health

Identification

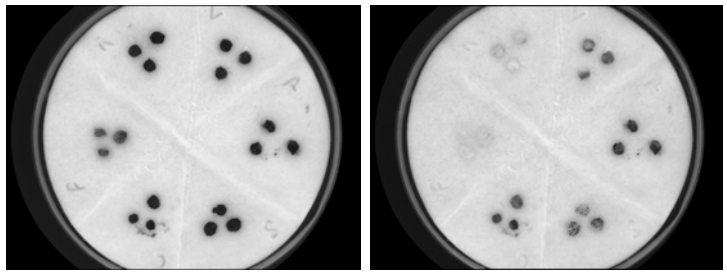
- Outside or inside the seed coat
- Microscopy, PCR, ELISA
- Colour, size and shape of the fungal structures

# Technologies in seed science and testing

- RGB imaging
- Hyperspectral and **multispectral imaging**
- X-ray imaging
- Magnetic resonance imaging
- (Infrared spectroscopy)
- **Near infrared spectroscopy**
- Chlorophyll fluorescence
- **Oxygen measurement (respiration)**
- Ethanol measurement (fermentation)

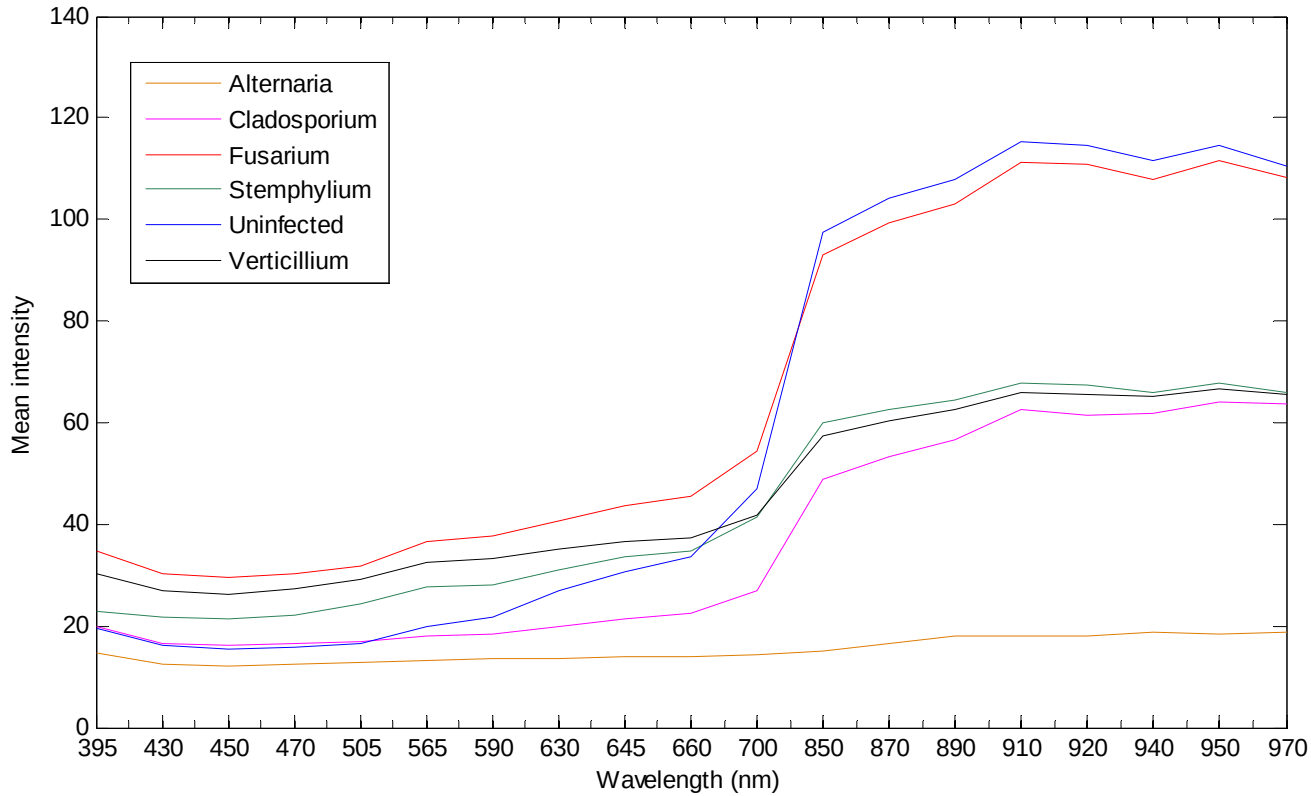
# Multispectral imaging

- 19 light emitting diodes
  - wavelengths ranging from 395 to 970 nm
- High-resolution camera
  - 1280 x 960 pixel images

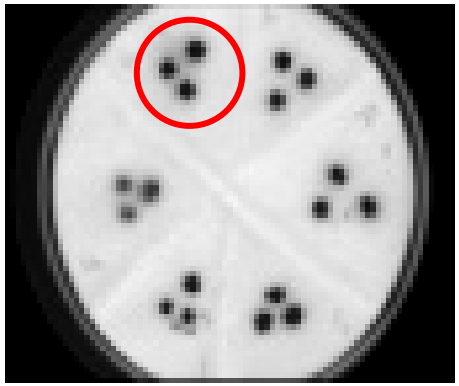


Infected spinach seeds

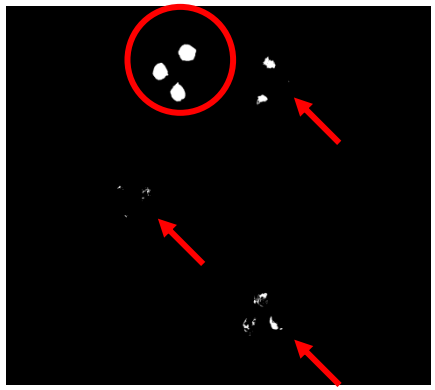

# Spectral signature of mean data



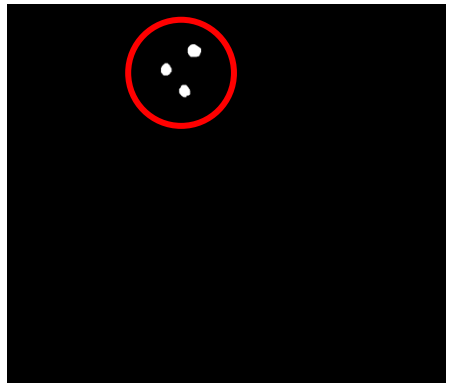
# Segmentation of uninfected seeds from infected seeds



Raw picture



CDA and treshold setting



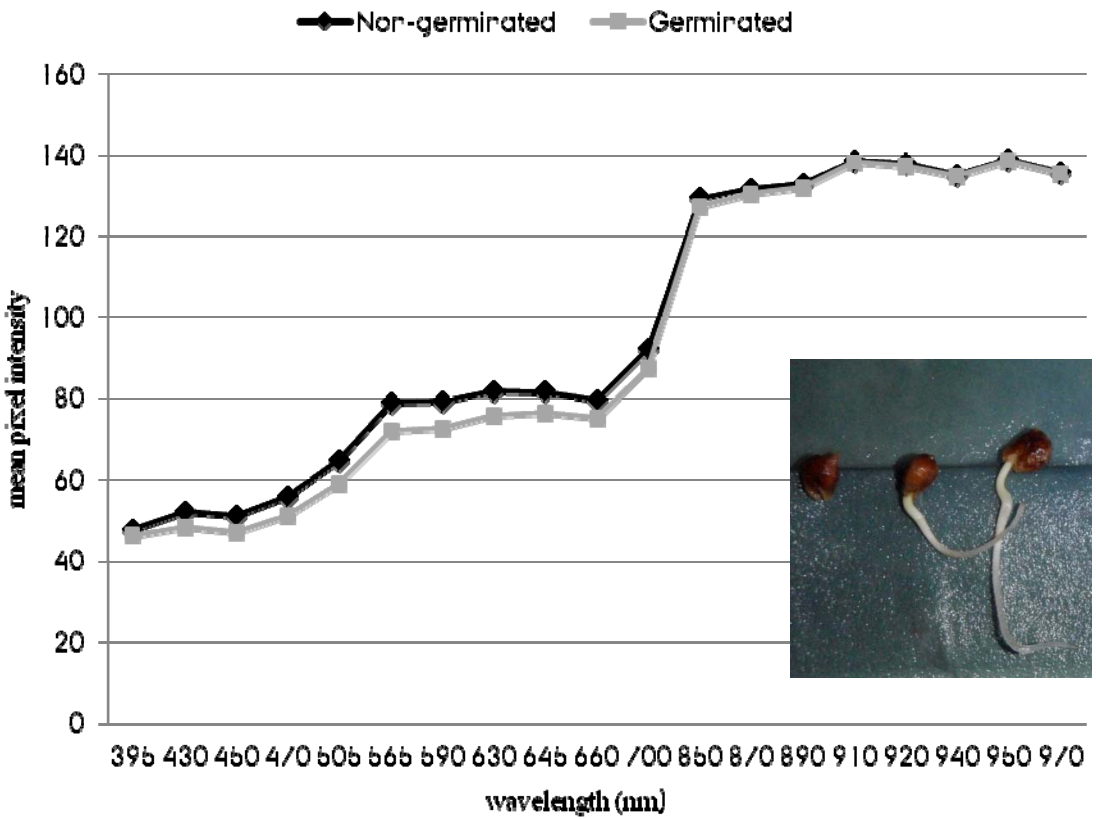
Binary morphology

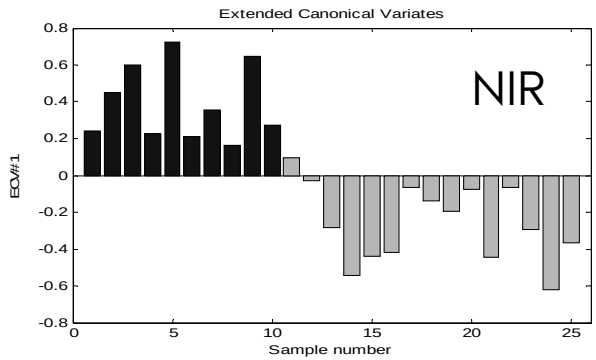
Test of 100 naturally infected seeds

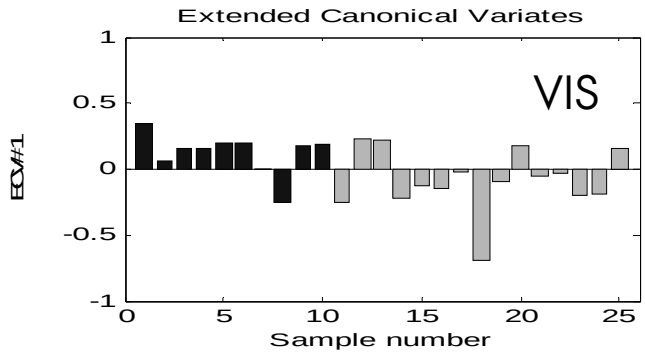
84% uninfected detected by VideometerLab

71% uninfected observed by microscope

# Multispectral imaging and germination



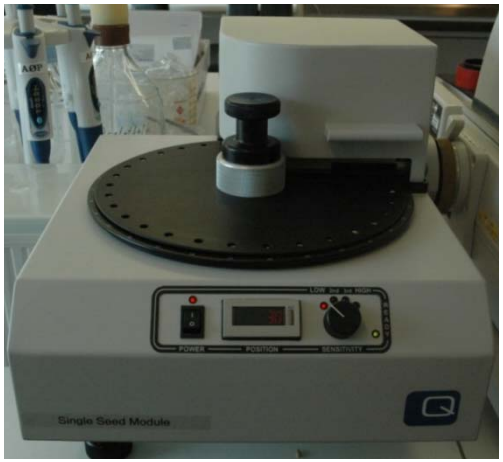



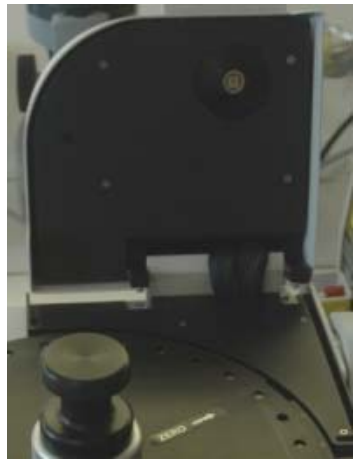




- Average of 300 seeds

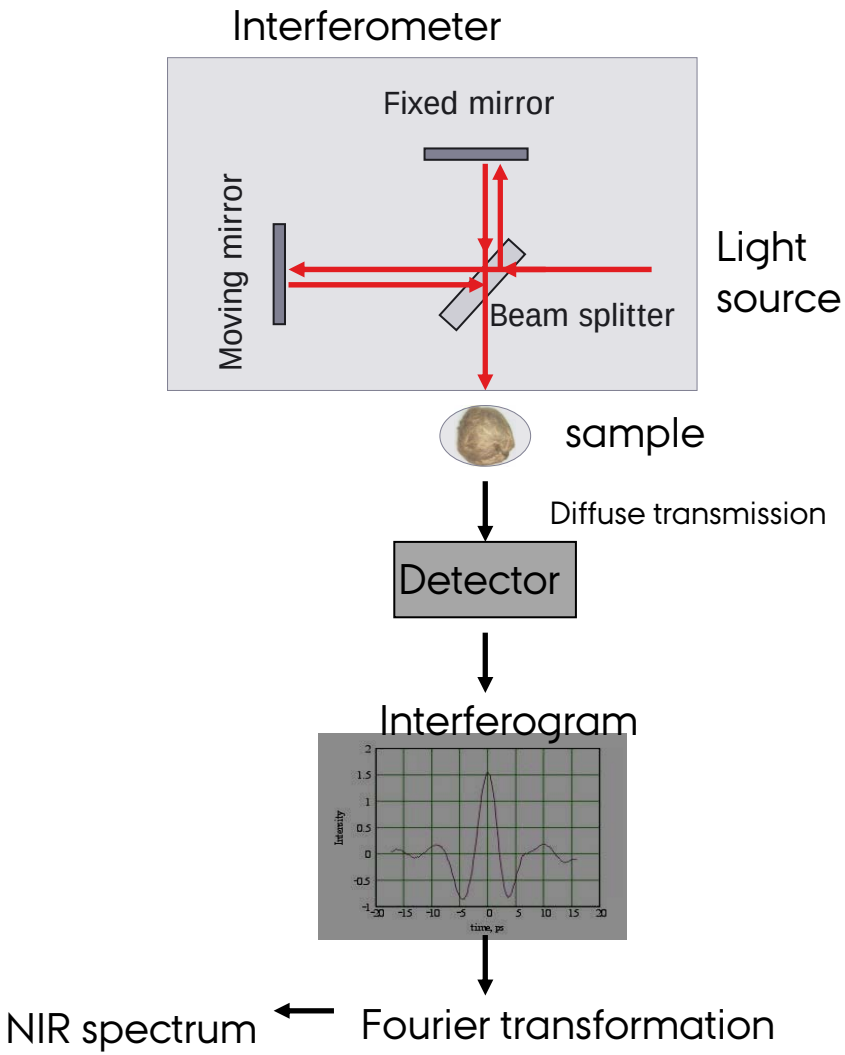
Pre-study of 25 seeds

# FT-NIR spectroscopy

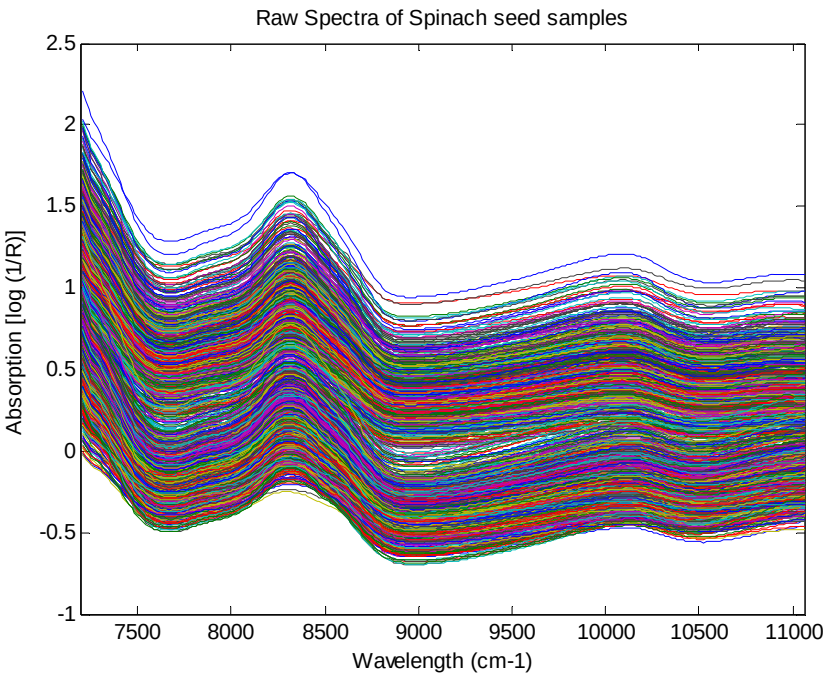


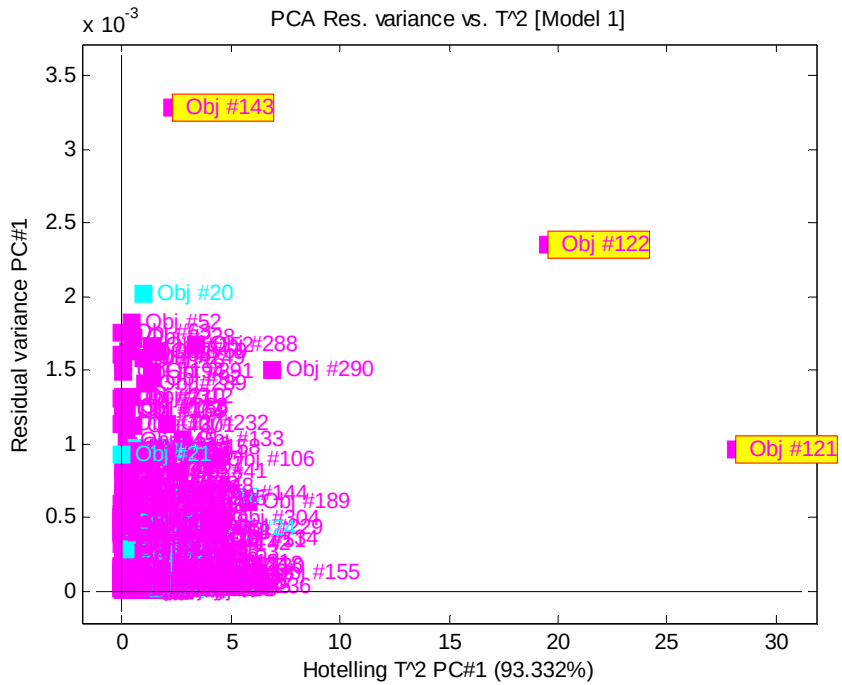



NIR spectrum ranging from 833 to1667 nm
30 seeds within 12 min



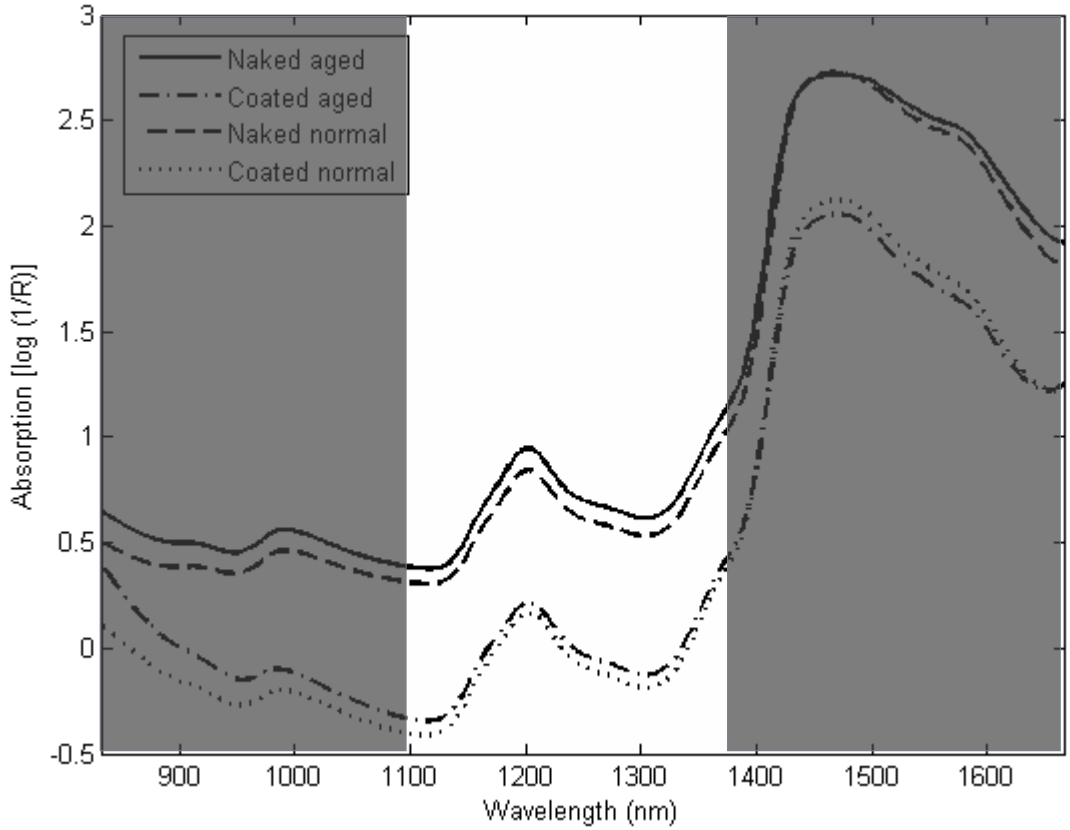
# Explorative evaluation




Outlier detection (PCA plot and Hotelling's T-square versus residual variance plots for PC1)

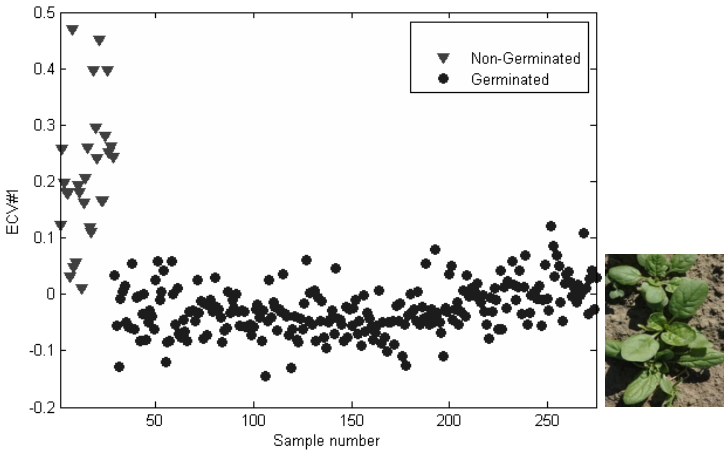
# Mean spectra



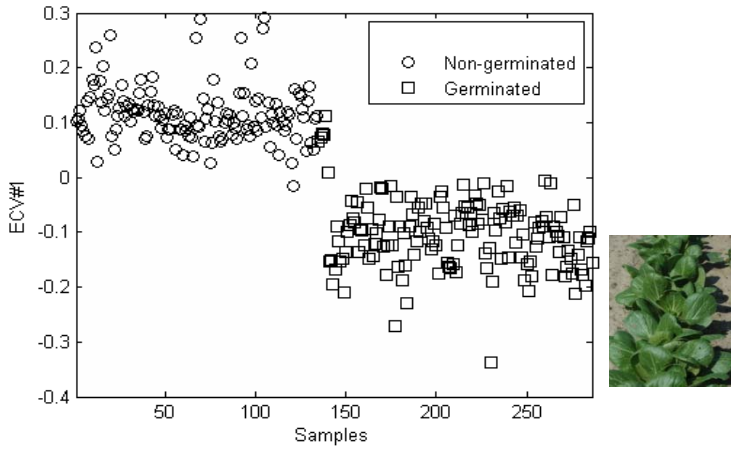
# Classification (ECVA = supervised model)

| Spinach seed germinated at 15°C | Germination % | Calibration set germ/non germ | Validation set germ/non germ | #PCs | Correctly classified seeds (%) |
|---|---|---|---|---|---|
| Normal, without pericarp | 99 | - | - | - | - |
| Normal, with pericarp | 97 | 175/2 | 108/2 | 9 | 93.6 |
| Aged, without pericarp | 86 | 150/12 | 127/8 | 10 | 91.1 |
| Aged, with pericarp | 87 | 150/15 | 108/9 | 8 | 98.3 |

# Scatter plot

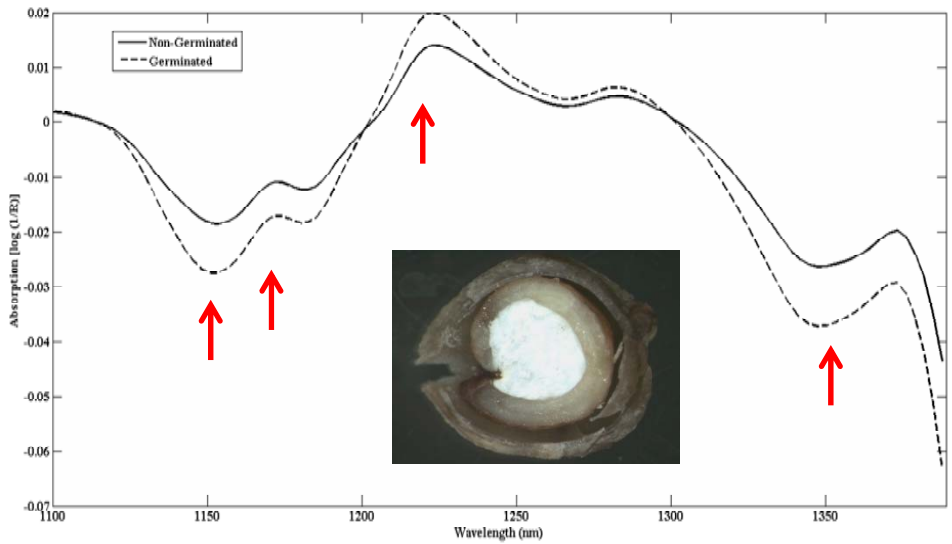



Spinach




Pak choi

# Assignments of NIR spectra




*NEAR INFRARED SPECTROSCOPY IN FOOD ANALYSIS*

**Table 2.4**—*continued*

| *Wavelength (nm)* | *Bond vibration* | *Structure* |
|---|---|---|
| 1037 | 2 × C—H str. + 2 × C—H def. + $(CH_2)n$ | oil |
| 1053 | 2 × C—H str. + 2 × C—H def. + $(CH_2)n$ | $CH_2$ |
| 1060 | N—H str. second overtone | $RNH_2$ |
| 1080 | 2 × C—H str. + 2 × C—C str. | benzene |
| 1097 | 2 × C—H str. + 2 × C—C str. | cyclopropane |
| 1143 | C—H str. second overtone | aromatic |
| 1152 | C—H str. second overtone | $CH_3$ |
| 1170 | C—H str. second overtone | HC=CH |
| 1195 | C—H str. second overtone | $CH_3$ |
| 1215 | C—H str. second overtone | $CH_2$ |
| 1225 | C—H str. second overtone | CH |
| 1360 | 2 × C—H str. + C—H def. | $CH_3$ |
| 1395 | 2 × C—H str. + C—H def. | $CH_2$ |

(Osborne et al., 1993)

# Conclusions

- Non-destructive technologies tested in my Ph.D project have proved to be valuable tools in correlation of colour and biochemical information to seed germination and health of spinach seeds

- Control of Verticillium in seed production fields is important and multispectral imaging can be employed as a fast seed testing method

- Both methods are user friendly, rapid and effiecient for testing the seeds

# Thank you

# Papers included in Ph.D thesis

Seed health

M. H. Olesen, J. M. Carstensen and B. Boelt (2010). Use of VIS-NIR system combined with multispectral image analysis in spinach seed. **Proceedings of the 14th International Conference on NIR Spectroscopy. 21-25**.

M. H. Olesen, J. M. Carstensen and B. Boelt (2011). Multispectral imaging as a potential tool for seed health testing of spinach. **Seed Science and Technology. 39, 140-150** .

M. H. Olesen, R. Gislum and B. Boelt (2012). Verticillium infected soil and seed treatment evaluation in spinach seed production. **Acta Agriculturae Scandinavica B. Submitted**

Germination

M. H. Olesen, N. Shetty, R. Gislum and B. Boelt (2011). VIS-NIR imaging – A tool to predict germination of spinach seed. **Proceedings of the 15th International Conference on NIR Spectroscopy. Submitted**

M. H. Olesen, N. Shetty, R. Gislum and B. Boelt (2011). Classification of viable and non-viable spinach (*Spinacia oleracea* L.) seeds by single seed near infrared spectroscopy and extended canonical variates analysis. **Journal of Near Infrared Spectroscopy, 19, 171-180.**

# Papers not included in Ph.D thesis

N. Shetty, T-G. Min, R. Gislum, M. H. Olesen, and B. Boelt (2011). Optimal sample size for predicting viability of cabbage and radish seeds based on NIR spectra of single seeds. **Journal of Near Infrared Spectroscopy, 19. 451-461.**

N. Shetty, M. H. Olesen, R. Gislum, L. C. Deleuran and B. Boelt (2011). Use of PLS-DA on VIS-NIR multispectral image data to examine germination ability and speed in spinach seeds. **Journal of Chemometrics. Accepted**

L.C. Deleuran, M.H. Olesen, N. Shetty, R. Gislum and B.Boelt (2011). Importance of seed Quality for the fresh-cut chain. **Acta Horticulturae. Accepted**