Microtomography and image processing tools for plant seed analysis: Current applications and future prospects

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Introduction

Conventional X-rays radiography has long been applied as a fast and non-destructive method in plant seed analysis. Applications have included damaged and/or abnormal seeds detection and evaluation [1,2,3] and two-dimensional measurements [4,5,6]. The major disadvantage of conventional X-rays seed radiography is that the information we intend to obtain is dependent on the position of the seed relative to the energy source. This limitation result on a lot of uncertainty particularly when attempting to couple X-ray data with other classical estimators of seed quality testing like germination potential and vigor. X-ray Microtomography, a technique that uses computer assisted X-rays to create virtual cross-sections of an object, has recently been successfully used to obtain high-resolution three-dimensional images of inner and outer structures of plant seeds and tissues [7,8]. In Microtomography, an approximated variation of density in each point of the sample is thus determined. The 3D volume of tissues with different densities can be separated or segmented using several techniques of image processing and huge data management. Herein, we present three new applications of Microtomography coupled with image processing for plant seeds analysis.

Applications of X-ray Microtomography to plant seeds

(1) Three-dimensional Measurements of internal and external beet seed structures:

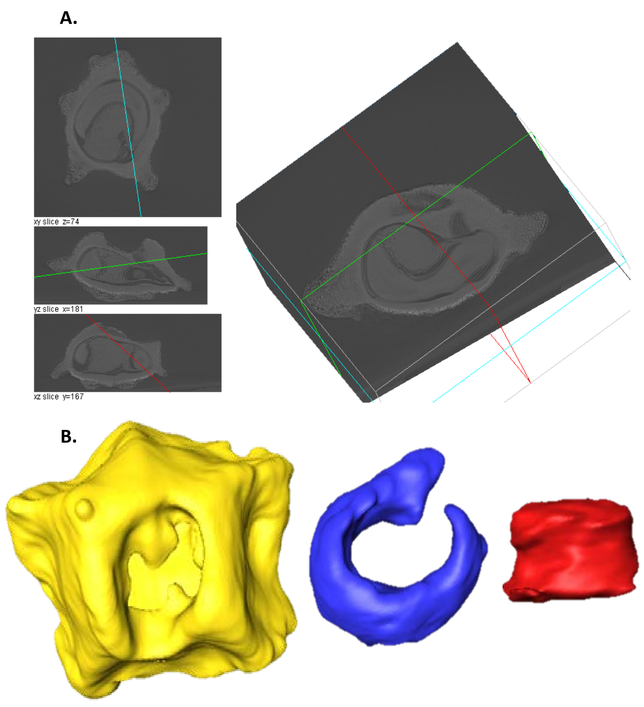

Figure1:
Segmentation of the sugar beet seed tomography images: (A) 2D slice views of sugar beet seed image , (B) 3D rendering of the separated structures: seed coat (yellow), embryo (blue) and pericarp (red).

Morphological seed characters may be used for the assessment of complex and quantitative traits such as seed resistance, germination potential and other agronomic traits. Examples for such direct measurements are image-based analysis of internal and external seed structures. For the AKER project, we are running an analysis of internal and external sugar beet seed structures using high-resolution tomography imaging (Fig1-A). The developed image processing pipeline allows 3D-volume and surface area measurements of three principal components namely: seed coat, embryo and perisperm (Fig1-B).

About AKER Project

AKER aims to improve the competitiveness of sugar beet by 2020 by doubling the annual increase in sugar yield / hectare (4% vs 2%).
AKER is part of the "Programme d'Investissements d'Avenir" and is supported by 11 public and private partners, representing the whole of the French sugar beet sector.
AKER is an original and innovative programme for research, development and training, confirming sugar beet as a crop and industry reference.

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AKER Betterave2020

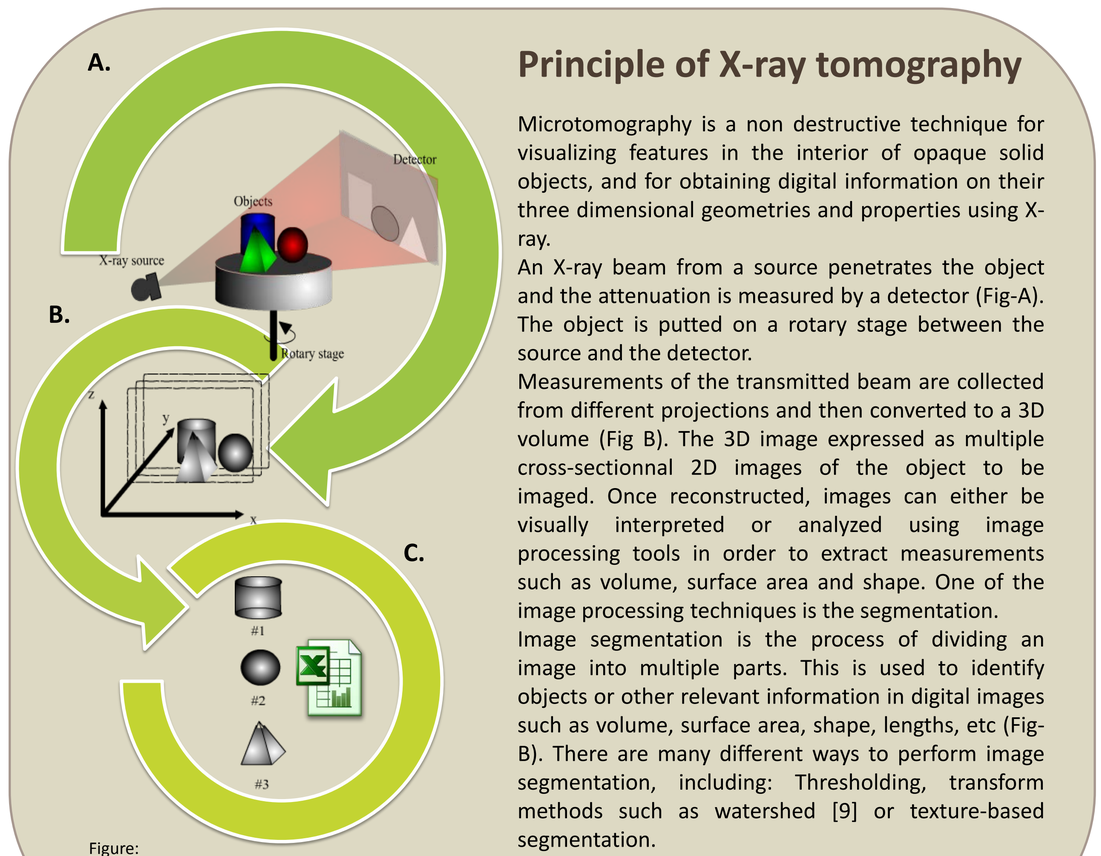

Figure:
Principle of the 3D X-ray tomography: (A) Scanning, and image acquisition (B) Image reconstruction, (C) image processing and analysis

Principle of X-ray tomography

Microtomography is a non destructive technique for visualizing features in the interior of opaque solid objects, and for obtaining digital information on their three dimensional geometries and properties using X-ray.
An X-ray beam from a source penetrates the object and the attenuation is measured by a detector (Fig-A). The object is putted on a rotary stage between the source and the detector.
Measurements of the transmitted beam are collected from different projections and then converted to a 3D volume (Fig B). The 3D image expressed as multiple cross-sectionnal 2D images of the object to be imaged. Once reconstructed, images can either be visually interpreted or analyzed using image processing tools in order to extract measurements such as volume, surface area and shape. One of the image processing techniques is the segmentation.
Image segmentation is the process of dividing an image into multiple parts. This is used to identify objects or other relevant information in digital images such as volume, surface area, shape, lengths, etc (Fig-B). There are many different ways to perform image segmentation, including: Thresholding, transform methods such as watershed [9] or texture-based segmentation.

(2) Volume assessment of seed and coating material on coated sugar beet seeds:

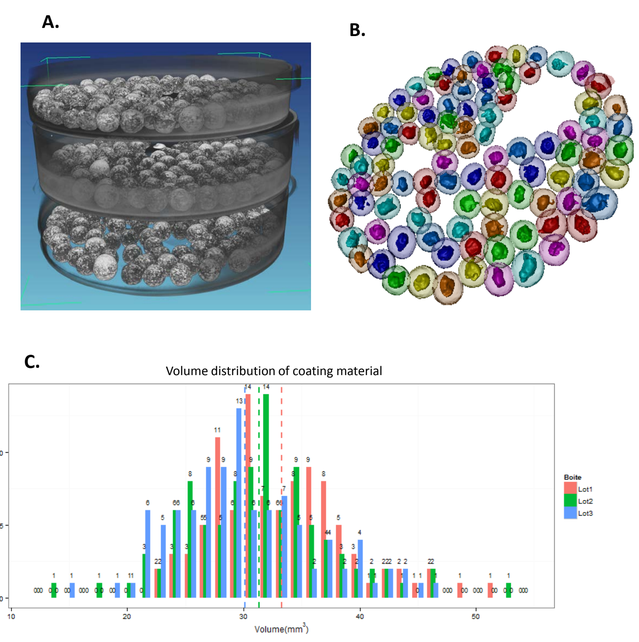

Figure2:
Separation and segmentation of coated sugar beet seeds: (A) 3D Raw image, (B) 3D of separated components, (C) Volume distribution for 3 lots.

Seeds are coated for seed sizing purposes, and protection against pathogens. Coated seeds are widely used as a standard product for many crops. The quality of the coating material may be evaluated using X-rays tomography.
The developed approach for coated sugar beet seeds allows to separate and extract from Microtomography images (Fig2-A) the volume of each seed, the volume of each coating material (Fig2-C).
The volume of each coating material was deduced from the difference between the total volume and the seed volume.
The results are expressed as the volume distribution of coating materials (Fig2-C).

(3) Three-dimensional characterization of insect damage in Faba bean seed:

About PeaMUST Project

PeaMUST aims at developing new varieties of peas and optimizing their symbiotic interactions to stabilize the yield and quality of pea seeds in the context of climate change and the reduction of pesticide use. To achieve this ambitious goal, PeaMUST benefits from an exceptional period of 8 years, a significant contribution from the main pea breeding companies and broad scientific and technical partnership.

For more information:
https://www.peamust-project.fr/

PeaMUST

Insects can cause internal and/or external damage by feeding on seed and this infestation can take place before or after harvest. X-ray 3D microtomography can be used as a non-destructive method to assess quantitative data of the damage. In order to assess the damage caused by insects in faba bean seed as a part of the PeaMUST project, we used microtomography and image processing. In the approach we developed, we made an estimation of the initial theoretical volume of the seed (assuming no damage) using convex hull [10] and the actual volume of the non-damaged area is subtracted from this estimation to give the volume of the damaged area.

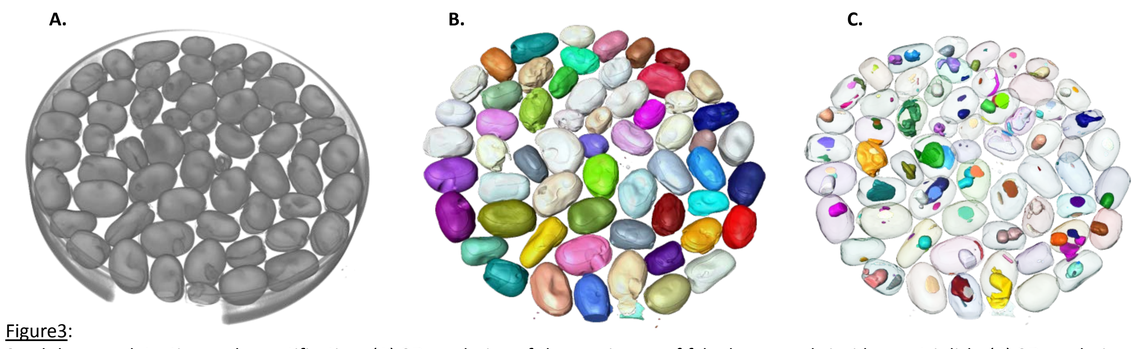

Figure3:
Seed damage detection and quantification. (A) 3D rendering of the raw image of faba been seeds inside a petri dish, (B) 3D rendering of initial theoretical seed shape (assuming no damage), (c) 3D rendering of the cavities resulted in insect damage.

Conclusion and perspectives

In this work, we attempt to show the relevance of Microtomography for both individual seed measurement with high-resolution (volume measurements of internal beet seeds morphologies) and for a batch of seeds (measurement of the coating material on coated seeds, and detection of seed internal damages). In comparison with the conventional 2D X-ray imaging, new traits can be now assessed using this 3D technology with a better resolution for each internal structures and the developed image processing allows automated detection with more precise and reproducible measurements.

Future prospects involve the increase of automation level of seed image acquisition and processing in order to attempt the challenge of high-throughput phenotyping. In addition, the possible use of Microtomography for detection and quantification of pathogens, priming effect, and imbibition process will be tested on different seed species.

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References

[1] Gustafsson, Å. & Simak, M. X-Ray Photography and Seed Sterility in Phragmites Communis Trin. Hereditas 49, 442–450 (1963).
[2] Machado, C. F. & Cícero, S. M. 'Aroeira-branca' [Lithraea molleoides (Vell.) Engl. - Anacardiaceae] seed quality evaluation by the X-ray test. Scientia Agricola 60, 393–397 (2003).
[3] Belin, E., Rousseau, D., Lechappe, J., Langlois-Meurinne, M. & Dürr, C. Rate-distortion tradeoff to optimize high-throughput phenotyping systems. Application to X-ray images of seeds. Computers and electronics in agriculture 77, 188–194 (2011).
[4] Gagliardi, B. & Marcos-Filho, J. Relationship between germination and bell pepper seed structure assessed by the X-ray test. Scientia Agricola 68, 411–416 (2011).
[5] Burg, W. J. van der, Aartse, J. W., Zwol, R. A. van, Jalink, H. & Bino, R. J. Predicting Tomato Seedling Morphology by X-ray Analysis of Seeds. J. Amer. Soc. Hort. Sci. 119, 258–263 (1994).
[6] Copeland, L. O. & McDonald, M. Principles of Seed Science and Technology. (Springer, 2001).
[7] Cloetens, P., Mache, R., Schlenker, M. & Lerbs-Mache, S. Quantitative phase tomography of Arabidopsis seeds reveals intercellular void network. PNAS 103, 14626–14630 (2006).
[8] Staedler, Y. M., Masson, D. & Schönenberger, J. Plant Tissues in 3D via X-Ray Tomography: Simple Contrasting Methods Allow High Resolution Imaging. PLoS ONE 8, e75295 (2013).
[9] Soille, P. Morphological Image Analysis: Principles and Applications. (Springer-Verlag Berlin and Heidelberg GmbH & Co. K, 2010).
[10] Barber, C. B., Dobkin, D. P. & Huhdanpaa, H. The Quickhull Algorithm for Convex Hulls. ACM Trans. Math. Softw. **22,** 469–483 (1996).

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