How can X-ray Microtomography be used to access seed morphology? Current applications and future prospects

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Introduction

Microtomography (micro-CT) is a non destructive technique that uses X-rays to investigate internal anatomy and morphology of organisms. It generates a series of projections reflecting the level of attenuation of X-rays after crossing an object from different angles during its rotation. Projections are then collected and reconstructed to obtain a 3D stack volume. Micro-CT is an emerging technique in plant seed science and technology [1,2,3,4] because of its ability to assess numerous seed structures (i.e. embryo, cotyledons, teguments, cavities, etc.) and quality (i.e. cracks, insect damage, defects, etc.) with a high accuracy (up to 5 micron resolution). Seed external appearance does not reflect the quality of the seed. Seed may seem intact while it can be damaged, cracked, have an abnormal embryo etc. The use of micro-CT can thus provide more information about seed quality. It can also be used for the characterization of varieties for different traits or for coated/treated seeds for the physical quality of the treatment. Here, capabilities of micro-CT coupled with image processing algorithms we developed are discussed for different applications on seeds.

Applications of X-ray micro-CT to plant seeds

Three-dimensional High-resolution Measurements of internal and external seed structures

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 use micro-CT and automated image processing pipeline to quantify several sugar beed seed structures from micro-CT images. The pipeline separates three morphological structures of the seed namely the embryo, the perisperm and the seed coat (figure1). Once separated these structures are analyzed separately in order to extract phenotyping quantitative traits such us volumes, shapes, length, etc.

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.

For more information:
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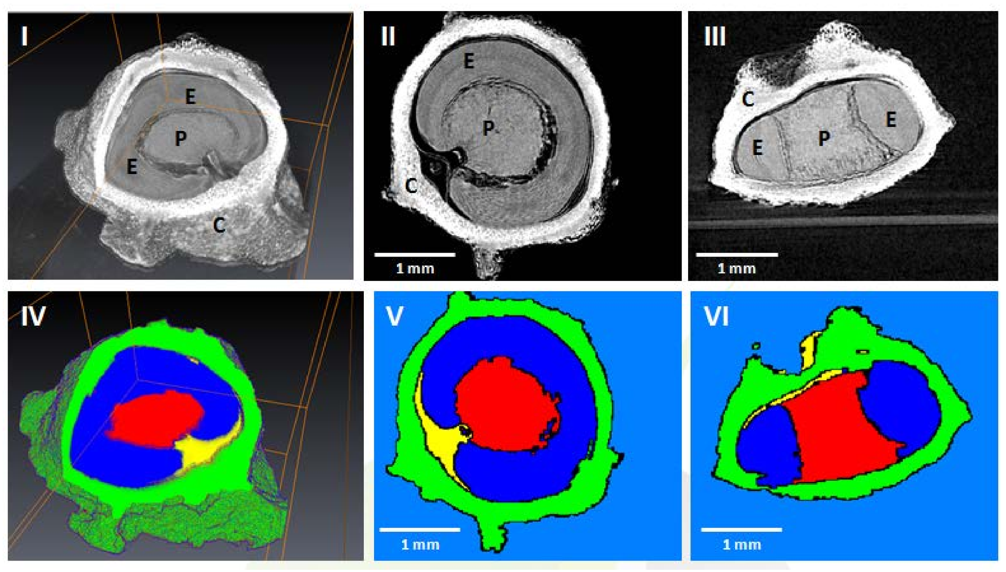
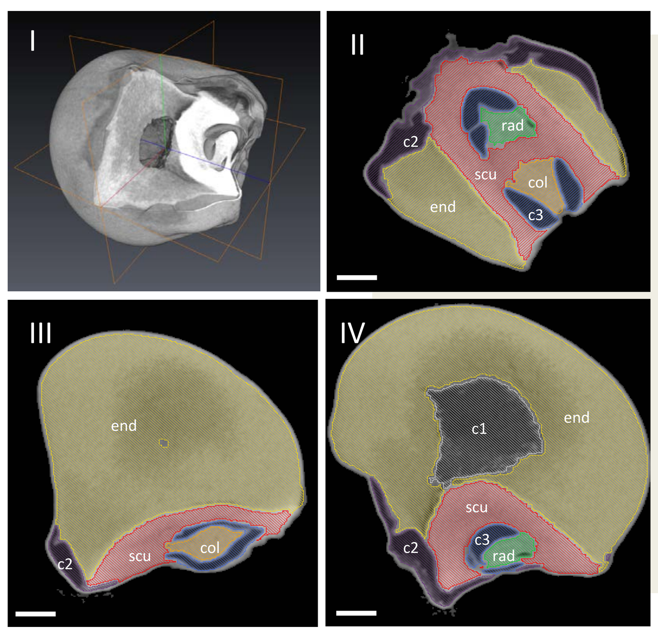


← Figure 1
Segmentation of high resolution beet seed images acquired with 3D X-ray microtomography. (I) 3D volume rendering with a corner cut showing the structural components of the seed namely E for embryo, P for perisperm and C for seed coat, and with respectively transverse (II) and longitudinal (III) cross-section views. The result of segmentation shows separated components with different labels (blue for embryo, red for perisperm and green for seed coat) respectively showed with a volume rendering (IV), transverse (V) and longitudinal (VI) cross-section views.
Scale bar = 1 mm

We also use micro-CT to study qualitatively and quantitatively the characteristics of the internal morphology of corn seeds. The developed image processing procedure is based on the detection of edges that separate different seed structures (endosperm, embryo and scutellum), and internal cavities. This procedure includes also the detection and quantification of cracks. This analysis addresses issues related to physical and germination seed quality, including industrial processes, treatment products, but also as part of the varietal characterization.



← Figure 2
High-resolution tomography of a corn seed (I) 3D Volume rendering showing the different corn structures. (II) , (III) and (IV) virtual cuts successively in XY , XZ , YZ planes after separation of internal structures showing the albumen (end) , the scutellum (scu) , the radicle (rad) , Coleoptile (col) and the cavity in the endosperm (c1) the gap characterizing seed filling (c2) and the embryo cavity (c3).
Scale bar = 1 mm

Principle of X-ray tomography

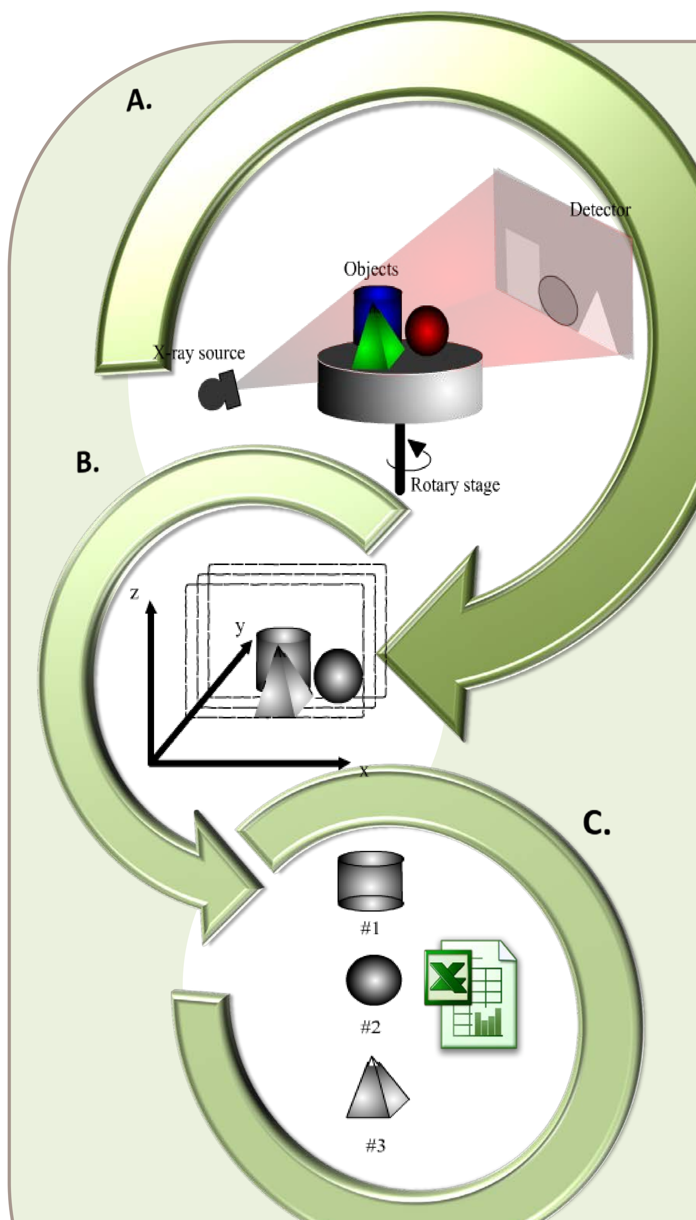


Figure:
Principle of the 3D X-ray tomography: (A) Scanning, and image acquisition (B) Image reconstruction, (C) image processing and analysis

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-section 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 relevant information. One of the most common image processing technique is 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-C). There are many different ways to perform image segmentation, including: Thresholding, transform methods such as watershed [5] or texture-based segmentation.

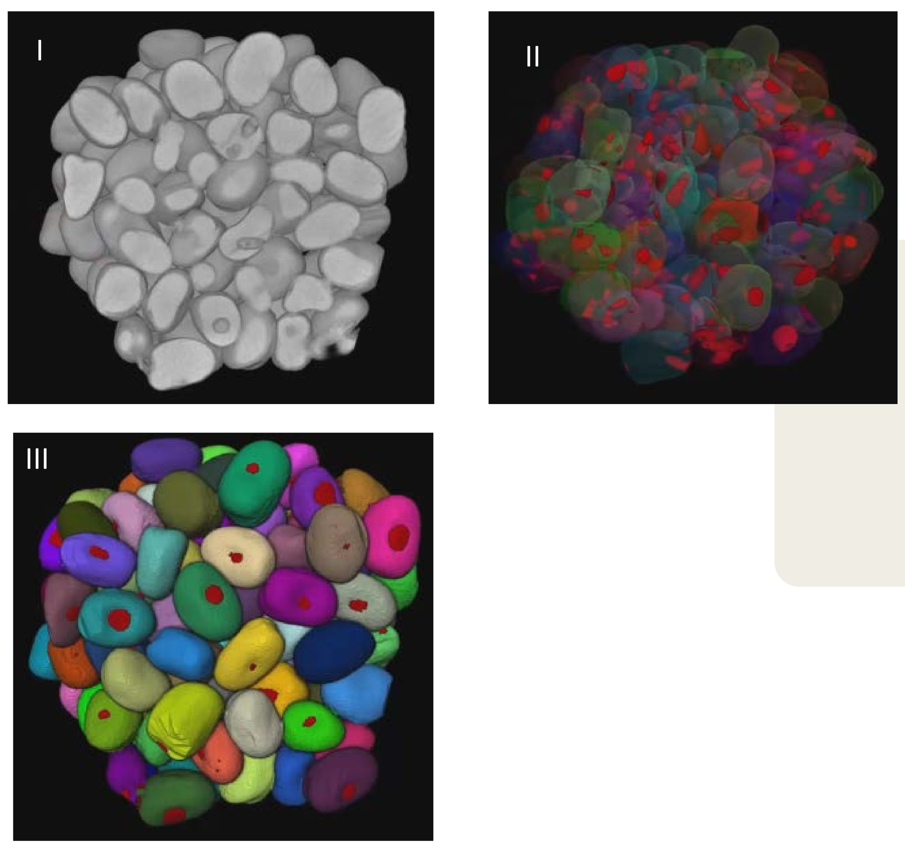
Insect damage detection and quantification

Insects can cause internal and/or external damage by feeding on seed and this infestation can take place before or after harvest. Damage caused by insects in pea and faba bean seed can be measured by micro-CT. In the approach we developed, we made an estimation of the initial theoretical volume of the seed (assuming no damage) using 3D convex envelope [6] of the initial seed shape. The actual volume of the non-damaged area is subtracted from this estimated volume to give the volume of the damage (figure 4).

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/



← Figure 4
Insect damage detection in faba bean seeds : (I) Raw image of a seed lot, (II) Image segmentation result showing individualized seeds and associated damage, (III) Damage visualization by seed transparency

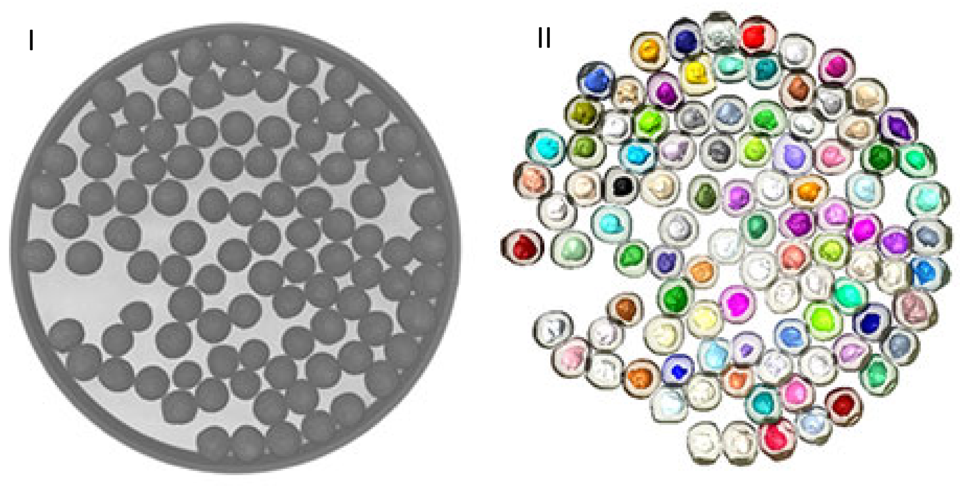
YouTube

Virtual removal of the pelleting material in coated seeds

Seeds are coated for seed sizing purposes, and protection against pathogens. Coated seeds are widely used as a standard product for many crops. The developed approach for coated seeds allows automated virtual of seeds and their coating material. The volume, the sphericity and the surface roughness are then evaluated for coated seed quality purposes. This approach is also used in seed purity for the detection of seeds of other species without destruction.

Figure 3 →
Sugar beet coated seed tomography . (I) Micro-CT image of a lot of coated seeds in a Petri dish . (II) segmentation of the image by separating coating material and seeds. The volume and surface area are extracted for each couple Coating/seed .

YouTube



Conclusion and perspectives

In this work, we attempt to show the relevance of micro-CT either in high-resolution for the quantification of internal seed morphologies (applications on sugar beet and corn seeds) or in medium-resolution and high throughput of a lot of seeds (applications on coated seeds, and insect damage on pea and faba bean. 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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Investissements d'avenir

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- to conduct DUS studies for the **Legal protection** of varieties (PBR)
- to evaluate the quality and the varietal identity of seed lots and for the **Certification** of seeds, for species requiring statutory certification.

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