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Automated seed testing by 3D X-ray computed tomography

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Abstract

In this paper, a fully automated CT-scanner is presented, which has been developed for testing of sugar beet seeds in industrial production environment. The system is simple to use, and no special training is needed for staff. The system has high throughput and the evaluation of volume images is performed automatically by dedicated image processing software. Quantitative measures of various characteristics of the parts of the seed (volume, thickness, weight) for each individual seed of a sample of 400 seeds are obtained. From these measurements, quality parameters can be derived to control seed processing.

Keywords: computed tomography, CT, image processing, seed production, seed testing, X-ray

Introduction

Computed tomography (CT) is a non-destructive inspection method, which is now in common use in medicine as well as in industry for development and critical production processes (Carmignato *et al.*, 2018). While the advantages of CT over X-ray radiography are clear (see below), CT is not in common use yet for seed production or breeding except for research (Gomes-Junior *et al.*, 2017). The main reasons for this are the high costs for the equipment, the complicated handling and low throughput of conventional CT scanners. Further, the CT images need to be evaluated, which is tedious work if performed by humans.

In this paper, a fully automated CT-scanner which has been developed for testing of sugar beet seeds in industrial production is presented (Porsch, 2015). The system is simple to use and no special training is needed for staff. The system has high throughput and the evaluation of volume images is performed automatically by dedicated image processing software. Several such systems have been in industrial use for many years now.

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Principles of X-ray computed tomography (CT)

X-ray computed tomography is an advanced technique compared with conventional X-ray radiography (Kak and Slaney, 1988; Buzug, 2008). While X-ray radiography provides only 2D images of the object, CT yields real 3D images with the full internal structure of the object under investigation. For a CT scan, many hundred X-ray radiographs are taken while the object is rotated (figure 1). This provides projection images from all sides of the object. With a special computer program, called reconstruction software, the 3D volume image (reconstruction) is calculated from these projections.

There are many factors influencing the quality of the reconstruction. For testing of small seeds, high spatial resolution in the order of 0.1 mm is required. A high precision CT scanner with microfocus X-ray tube, high-resolution detector and high precision mechanics is needed to achieve this high resolution. High resolution also means a large number of radiography images in order to capture all the details of the structures. But more images also mean more time for the scan. The challenge is to find a good compromise between image quality, throughput and costs.



Figure 1. Principle of X-ray computed tomography scanning.

Comparison of computed tomography and radiography for seed testing

X-ray radiography has been used for a long time in seed inspection. Widespread usage started in the 1960s (Bruggink and van Duijn, 2017). Nowadays, digital flat-panel X-ray detectors are in use and the inspection is usually done visually on a PC screen. However, the visual inspection of several hundreds of seeds per sample is a very tedious task. Moreover, the radiograph is a projection image only which gives no information about thicknesses, volume or depth.

In contrast, computed tomography provides full information of the interior of individual seeds (figure 2). This allows the quantitative measurement of volume, thickness, length, diameter and even mass for each part of the seed. In order to take advantage of this option, a specialised 3D image processing software is required to perform this complex evaluation in a reasonable time.

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Figure 2. Comparison of radiography (top left) and a computed tomography section (top right) of a sugar beet seed. Bottom: 3D view of high-resolution computed tomography of a sugar beet seed.

Description of the system

The CT scanner

The CT scanner is a stand-alone instrument the size of a small cupboard (figure 3). The system has a 50 μ m microfocus X-ray tube and is operated at 45 kV. The radiographs are caught by a one mega pixels X-ray flat panel detector with 200 μ m pixel pitch. The resulting spatial resolution is in the order of 50 μ m. The electronics for X-ray and robot control is in the lower compartment of the cabinet, as well as the computers for process control, image processing and data storage. Also, in the lower part is a pull-out cabinet which can take up to five sample trays (figure 3). An internal elevator can lift the trays in the processing position. A small internal Cartesian robot picks up the samples from the tray in processing position and places them on the small rotation stage of the CT scanner.

Sample processing and CT scan

There is a separate station for sample preparation and registration (figure 3). For conventional X-ray inspection by radiography, special preparation of the seeds is required. Each single seed must be placed in a separate hole of a special well plate to ensure that the seeds do not overlap and lay in the right orientation. This preparation is not required for CT scans. The seeds are just filled into small sample containers which are then placed on sample trays with wells. Several trays may be prepared during the day and stacked in the pull-out cabinet for overnight measurement.



Figure 3. View of the automated computed tomography (CT)-scanner (top) with pull out cabinet for the sample trays (bottom left) and sample preparation station (right).

When the tray shelf has been prepared, the sample processing can be started. All the steps necessary for the CT scan are done by the system autonomously. This includes conditioning of the X-ray source and calibration of the detector. The internal elevator brings up one sample tray after the other and the robot places all the samples one by one on the rotation stage for the CT scan. A scan including sample change takes about three minutes; 300 samples can be processed within 15 hours without any user intervention. The system is self-monitoring and can operate unattended. Reference samples can be used to control the performance of the system from time to time automatically. If a reference sample fails the test, the system stops further processing.

Image processing

The image processing takes place in parallel to the scan of the next sample. The first step of the image processing is the reconstruction of the 3D volume of the sample (figure 4). Then, the individual seeds are identified and separated (figure 5). Then the seed images are segmented into the different parts of the sugar beet seeds: soft and hard pericarp,

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true seed and free space. Finally, the characteristic dimensions are determined for each part, including volume, diameter and thickness. Even the mass of the different parts can be obtained from the CT image after calibration, since the X-ray density (= gray value) is proportional to the mass density for a given material. The challenge for the image processing is that seeds are natural products and as such have a high variability in shape and size. This is completely different from technical products. Using advanced computation techniques, the image processing of this system is keeping pace with the scan process.



Figure 4. Computed tomograph image of a sample with seeds with virtual cut to show the interior.



Figure 5. (A) Cross-section of the computed tomography image. (B) Separated and marked seed fruits. (C) Equatorial cross-section of a single seed sugar beet fruit. (D) Segmentation of the seed fruit with red colour = true seed (embryo + perisperm), green = free space, yellow and blue = hard and soft pericarp, respectively.

Outcome

In routine operation, the system provides a report with the mean values of all the relevant dimensions of the seeds for each sample. This information can be used for process control in seed processing. However, the system may report additional information for particular purposes. It can provide a full list of the individual quantities for each seed of a sample. This allows the calculation of variances and distribution functions. Furthermore, it can generate a set of 3D images of the individual seeds. This permits the trace down of every measure to each seed and special investigations of single seeds. Specific tools assist in this evaluation. The system can also provide a special table with images of equatorial cross-sections for all the seeds of a sample. This image table is useful for visual inspection for specific characteristics of seeds. An additional tool supports the labeling of seeds with special characteristics (figure 6).



Figure 6. Tool for visual inspection of cross-sections of the seeds. The highlighted fruit (#155), which is also displayed enlarged in the right window, is a twin fruit with two seeds within the fruit.

Sample results

The reproducibility of the method has been checked by a large number of repeated measurements. Before each scan the sample was shaken to mix the seeds. Table 1 gives a typical result for some relevant dimensions for repeated measurements of a sample with 401 sugar beet seeds. Except for free space, the deviations are below 1%. The reason for the stronger variations in free space volume are the many small gaps which partly have

Table 1. Values from three repeated measurements (A, B, C) by computed tomography of a sample with 401 sugar beet seeds: mean values for total volume and mass of the seeds (V_{total} , m_{total}), volume and mass of the true seeds ($V_{trueSeed}$, $m_{trueSeed}$), volume of the free space inside the seed cavity ($V_{freeSpace}$), minimum thickness of the perikarp ($d_{pericarp}$), and diameter of circumscribed sphere¹ of the true seed ($d_{trueSeed}$). In addition, mean value, standard deviation and coefficient of variation (CV) for the three repeated measurement are given.

	V _{total} (mm ³)	m _{total} (mg)	V _{trueSeed} (mm ³)	m _{trueSeedl} (mg)	V _{freeSpace} (mm ³)	d _{Pericarp} (mm)	d _{TrueSeed} (mm)
А	11.5	2.66	3.76	0.941	0.335	0.518	2.731
В	11.3	2.68	3.74	0.947	0.398	0.509	2.763
С	11.3	2.69	3.76	0.953	0.382	0.512	2.758
Mean	11.4	2.67	3.76	0.947	0.372	0.513	2.751
StdDev	0.07	0.02	0.01	0.006	0.033	0.005	0.017
CV	0.6%	0.6%	0.3%	0.6%	8.8%	0.9%	0.6%

¹ The circumscribed sphere is the smallest sphere containing the object (here the true seed).

thicknesses below one or two pixels $(50...100 \ \mu\text{m})$ and are therefore close to the detection limit. However, the total volume of free space for this sample is only 3% of the total volume of the seeds.

The volume of the true seed and the filling degree of the seed fruit are important measures of quality in sugar beet seed production (TeKrony *et al.*, 1969). The filling degree is the ratio of the volume of the true seed to that of the fruit cavity. As an example, figure 7 shows the results for four different samples. In samples B and C, the fruits have a very high filling degree while in samples A and D there is a considerable part of seeds with low filling degree (figure 7A). This is also reflected by the high number of small seeds in samples A and D (figure 7B).

Outlook

Automated CT scanners as described here are a reasonable alternative to the conventional testing of seeds by visual inspection of radiographs. The handling of the system is very simple, and no special sample preparation is required. Quantitative and reproducible results, including full documentation, are delivered for important quality measures like filling degree and true seed size. Because of the short processing time (three minutes), the system can be used for process monitoring. Due to the high capacity it is also useful for screening in breeding or research. While the system described here is designed for testing of sugar beet seeds, such systems can also be used for testing other kinds of seeds and fruits (figure 8).

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Figure 7. Examples of results obtained for four different samples of sugar beet seeds by evaluation with the computed tomography scanner: (A) distribution of filling degree; (B) distribution of true seed volume.

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Figure 8. Cross-section of computed tomography images from samples of seeds of (A) watermelon, (B) tomato and (C) walnut.

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