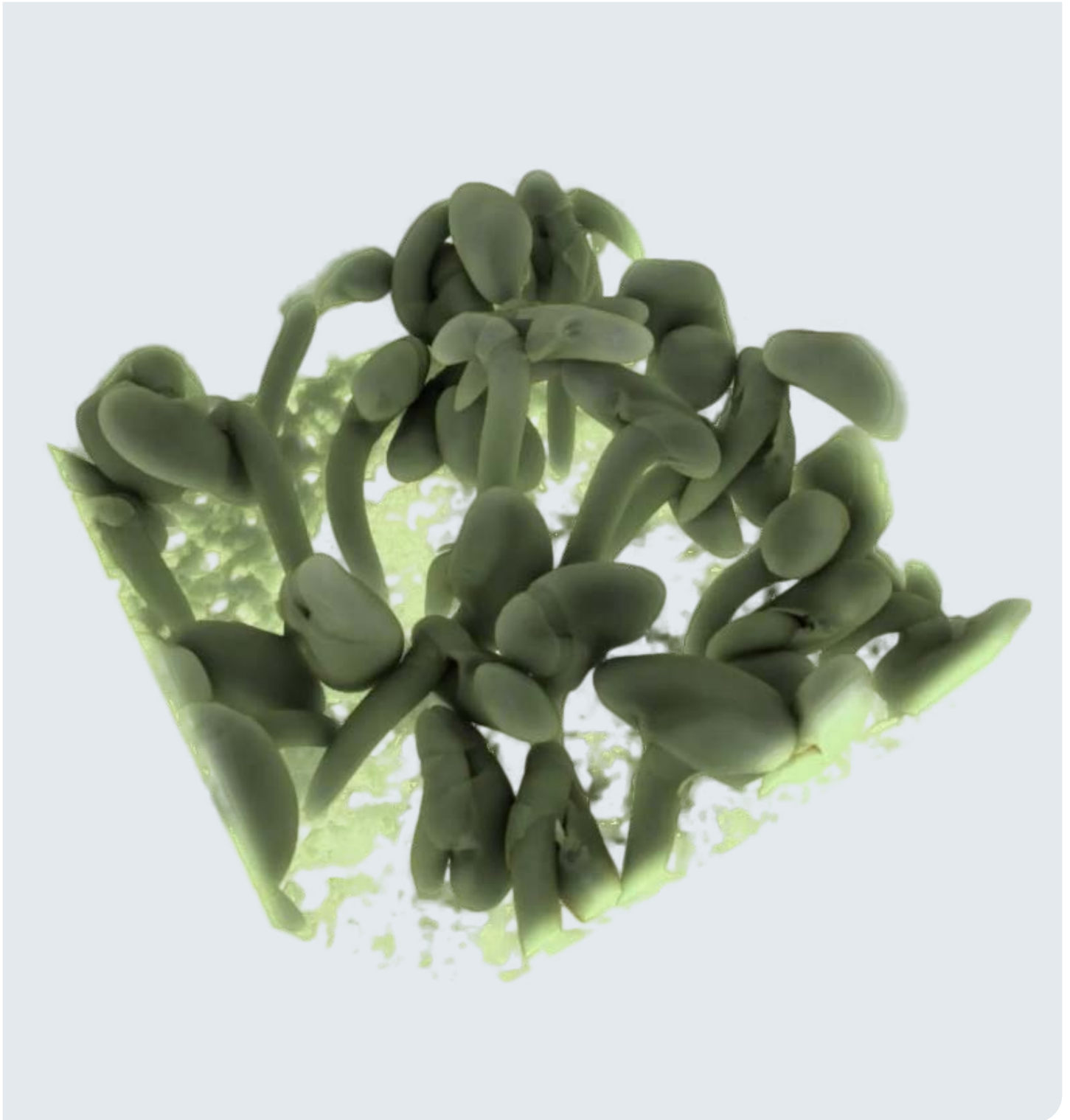# GERMINATING SEEDS

Opportunities for dynamic CT imaging in the laboratory

Germinating seeds: opportunities for dynamic CT imaging in the laboratory

TESCAN XRE, Ghent, Belgium. / Dynamic CT in the laboratory

Dynamic and time-lapse CT with X-rays has rapidly emerged as an essential technique to understand material evolution, facilitating in situ investigations ranging from mechanical deformation to fluid flow in porous materials and beyond. Imaging of dynamic processes is one of the key applications at synchrotron facilities, pushing the time resolution more and more down with quite some success.

In the laboratory, image quality and spatial resolution have been significantly improved, often at the cost of temporal resolution however. Recent developments at TESCAN XRE have made it possible to visualize and inspect dynamic process in the laboratory with a temporal resolution below 10 seconds. In this study we explore the challenges and innovations that have led to this capability. Two different scan approaches: time-lapse CT and dynamic CT will be explained during an examination of germinating seeds.

Introduction

As a non-destructive technique, micro-CT is an optimal technique to take repetitive scans of the same sample over time. The combination of taking different scans over time is called dynamic or time-lapse CT. By doing so, the scans can demonstrate how materials form, deform and perform over time or under certain external conditions. On the one hand, during the time interval, external parameters can be manipulated by using in-situ stages such as pressure or temperature stages. On the other hand, the sample itself can form or deform on its own (swelling, drying, growing, etc.).

The popularity of dynamic CT is drastically increasing, but still not many people are performing dynamic experiments on a regular base. This can be explained by the fact that performing dynamic experiments has some practical challenges during every step of the process (acquisition, reconstruction, visualization and analysis). Dedicated 4D designed workflows are needed to handle the complete workflow from acquisition to the analysis.

In this study, different approaches for both dynamic and time-lapse CT will be discussed. Dedicated hardware and software tools are being developed at TESCAN XRE to facilitate the execution of dynamic CT experiments in an intuitive and streamlined workflow.

## Different approaches time-lapse and dynamic imaging

Many different approaches of time-resolved imaging exist, each with its own challenge. A distinction can be made between time-lapse and dynamic.

Time-lapse imaging case corresponds with a step and shoot approach. A series of projections is taken over a specific scan time interval. The scan time interval can be chosen based on the speed of the process and can be in the orders of seconds up to several hours.

When working with in situ stages, the application of external parameters can be stopped during acquisition to obtain a stable situation during the scan time interval. However, stopping the external parameters can lead to unwanted processes inside the material (e.g. relaxation effect when stopping external load). In-between the different scan time intervals, a scan pause is inserted and may vary from seconds to days, depending on the process under investigation.

By using kinematic mounts, the sample can be removed from the scanner and reinstalled in the exact position inside the system to execute the actual scans. This is typically the case during longer time-lapse experiments or if the sample needs specific external conditions in between the scans.

To summarize, time lapse-scans are a series of individual scans over a specific time period
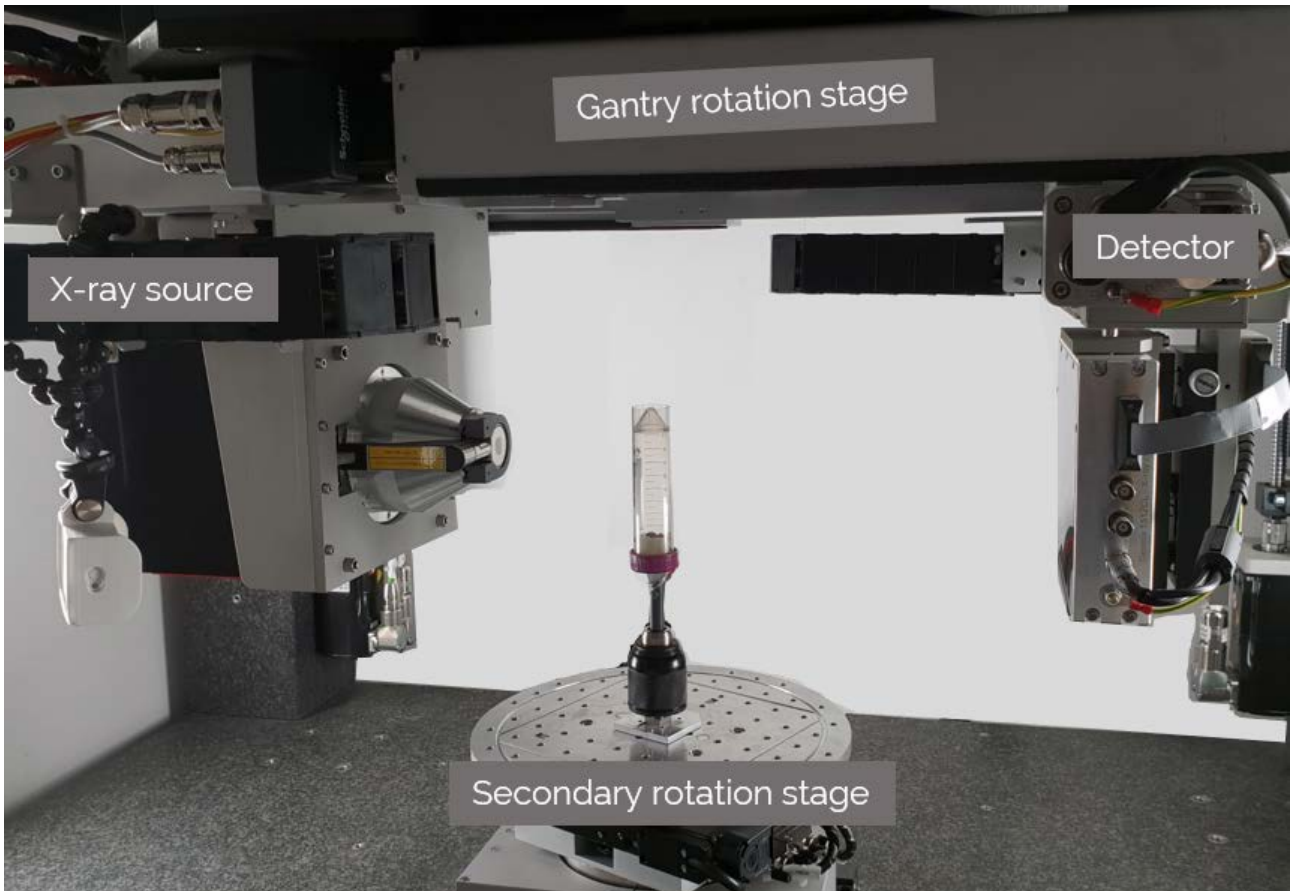
▲ **Fig 1:** Internal view of the DynaTOM with a gantry based rotation stage and fixed sample position (with an optional secondary rotation stage). X-ray source and detector are rotating around the sample.

During a dynamic experiment, there is one scan time interval during which an uninterrupted acquisition is taken. The scan time interval can be from minutes to a couple of hours or even days. During the uninterrupted acquisition, projections are taken, starting from zero degree up to tens of thousands degrees. The relative rotation of the sample in-between the X-ray source and detector is continuous and results in a multi-turn acquisition over the scan time interval, without going back every time to the initial position.

Time-lapse and dynamic acquisitions generate vast amounts of raw projection data, which need to be reconstructed, further post processed and eventually quantified. It is therefore essential to devise workflow strategies to quickly identify the interesting moments prior to reconstruction to optimize the amount of data that is generated, but also incorporate the added time dimension in the 3D analysis workflow to improve image quality. Different workflows of time-lapse dynamic micro CT imaging will be demonstrated here on germinating seeds.

DynaTOM system

The scans of the germination process of the seeds were obtained by using the DynaTOM system. This unique system is gantry based, with a fixed sample and horizontal rotation of the X-ray source and detector. This design is ideal for in situ, dynamic experiments. Temporal resolution of the system can go below 10 seconds. The design is such that continuous acquisition or rotation is possible without going back to a reference position. The system is equipped with a 130 kv/35 W X-ray source and a CMOS flat panel detector (1900 x 1500 px). Spatial resolution can go down to 3 µm. In addition, source-object and source-detector distances can be changed to optimize the flux and thus quality of the images. With the XY micro- positioning stage, the sample can be moved in a controlled way within the coordinate system of the scanner. This unique system is ideal for both a multi-scale and multi-temporal resolution for in situ experiments (figure 1).

Germinating seeds

To validate the possibilities and workflows of the time-lapse and dynamic approach, part of a germination process of cress seed has been imaged. Cress is generally quick and reliable to germinate and takes up to 10 days to fully grow.

Seeds need the proper environment (moisture, air, temperature…) to germinate. Generally, as the seed is taking up water, it starts to expand and the seed coating cracks. The root emerges from the seed and after absorbing water by the root, the shoot begins to emerge from the seed. Many studies are performed on the understanding of the sprouting process and the complete deformation of the 3D structure over time would tremendously contribute to the understanding of this complex process.
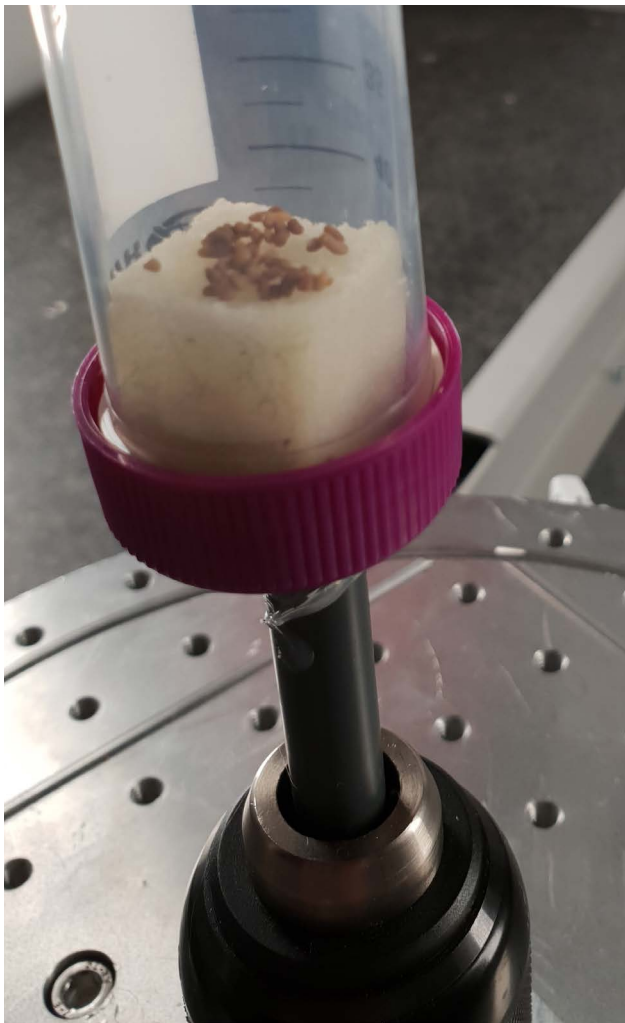
▲ **Fig 2:** Set-up of the experiment. Seeds were placed on a wet surface inside a plastic container and mounted inside the DynaTOM.

Experiment

The aim of this experiment is to obtain both high temporal and high spatial resolution volumes of germinating seeds and contribute to the understanding of the complex germination process.

The cress seeds were placed on a wet tissue inside a plastic container. The container itself was completely sealed to prevent drying during the experiment. To demonstrate both the multi-scale and multi-temporal approach, two scan approaches will be discussed.

Time-lapse imaging

The seeds were imaged over a total period of 130 hours. In total 100 scans were taken with a 1 hour interval in between 2 scans. A single acquisition time took 5 minutes over 360°. After each scan, the source and detector were positioned back at the 0° position. The scans were taken with a 2 x binning mode and resulted in an image dimension of 968 x 768 pixels. In total 1300 projections were taken with a single exposure time of 110 ms. The reconstructed voxel size was 20 µm. This type of acquisition resulted in 100 different projection folders. A schematic overview of the time-lapse approach is shown in figure 3.
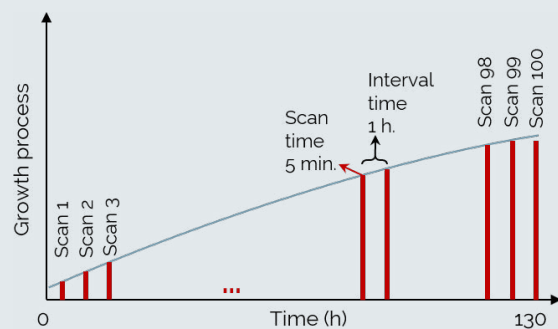

▲ **Fig 3:** Schematic overview of the time-lapse imaging approach. 100 scans were taken with a 5 minute acquisition time. In-between two scans, a 1 hour pause was installed.

Dynamic imaging

During a second approach, only 3 seeds were imaged. One continuous scan was taken for 276 minutes during the early stage of the germinating process (starting after 56 hours when placing the seeds on the wet tissue). The source and detector were rotated from 0° to 24840° in a single smooth movement, without going back to the reference position in-between a full rotation.

One full rotation lasted 4 minutes and also in this case the image dimension was 968 x 768 pixels. The reconstructed voxel size was 9 μm, because the source was placed closer towards the sample. This acquisition resulted in a single acquisition folder with 91,000 projections. A schematic overview of the dynamic imaging experiment is shown in figure 4.

69 individual reconstructed volumes are created during the reconstruction (see section reconstruction).
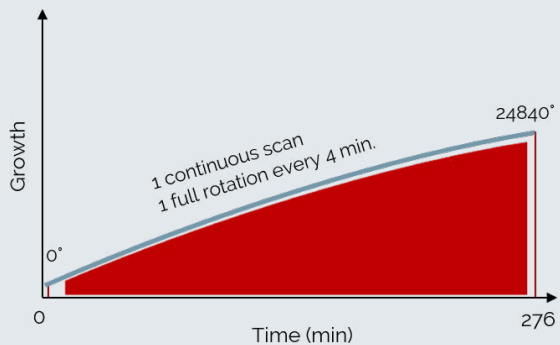

▲ **Fig 4:** Schematic overview of the dynamic imaging approach. One scan was taken over 276 minutes going from 0° to 24840° in a smooth continuous rotation. One full rotation lasted 4 minutes.
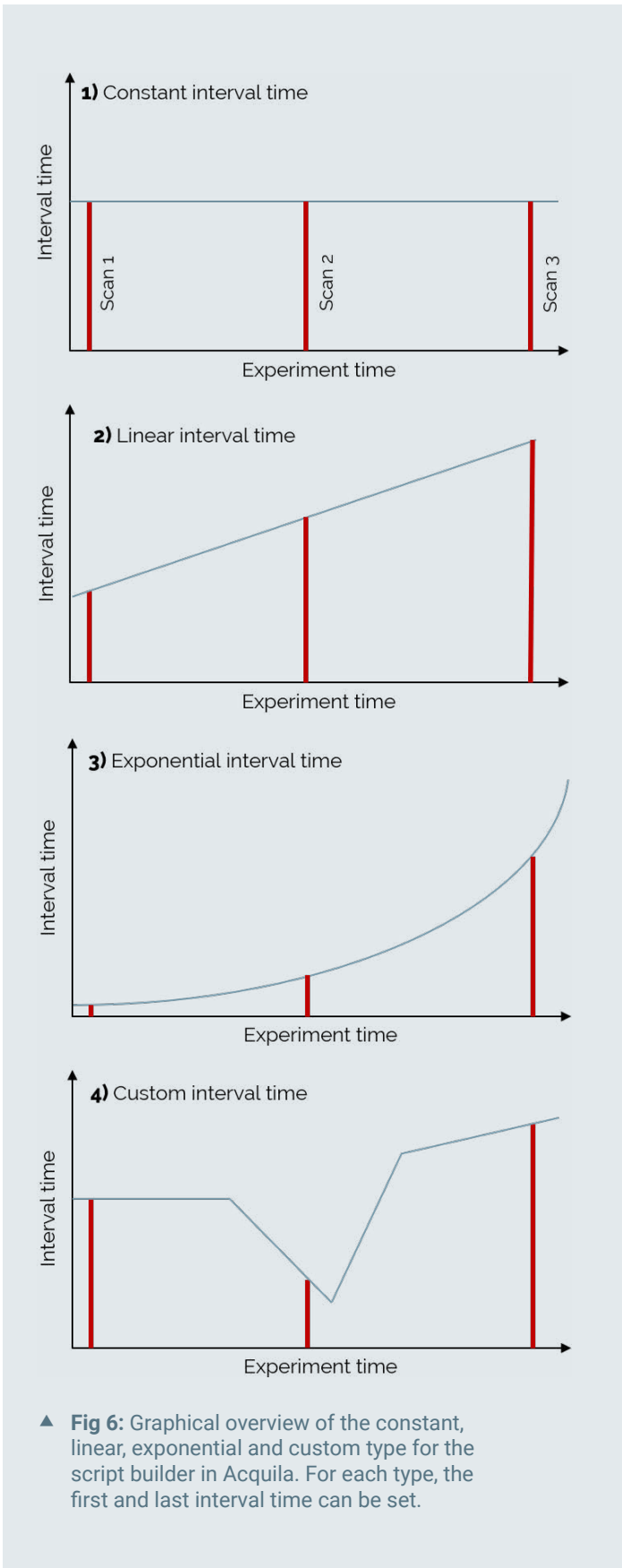
Acquisition software: Acquila

Acquila is the modular software architecture for image acquisition of the micro-CT systems from TESCAN XRE. During the scan preparation step, a user editable script is prepared by adding individual timesteps or a batch of timesteps. For the time-lapse preparation a user friendly script builder is available (figure 5). Depending on the dynamic process, four different approaches are implemented in the software; a constant, linear, exponential or custom interval. The four types are graphically shown in figure 6. In this study, the constant approach was implemented.

As a result of the script builder in Aquila, n number of scans will be batched in a single script. No further user interaction is needed during the execution of the script. The X-ray on/off command will be automatically enabled during the intervals. If needed, tube optimization protocols will be automatically executed during the intervals (depending on the available interval time).

The acquisition steps for the dynamic experiment are different, because one single scan is taken. The only scan parameter that needs to be tuned is the last angle. All possible pauses will be excluded from the script, including the 'move to reference position' after each full rotation (360°). As a result, a smooth uninterrupted multi-turn rotation will be performed.
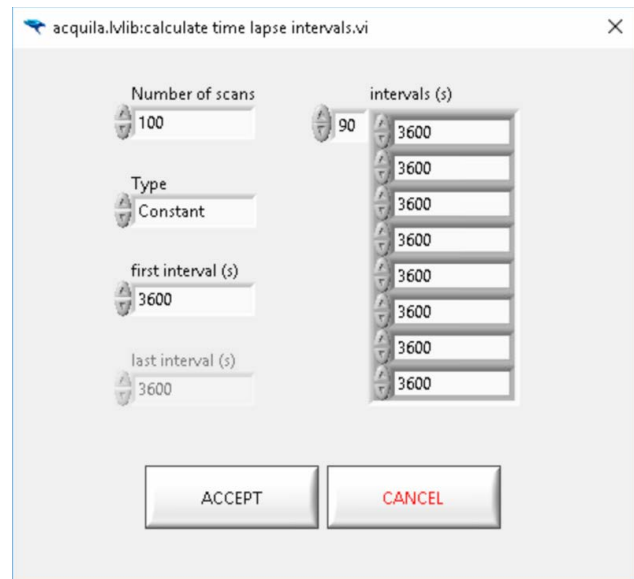
▲ **Fig 5:** Time-lapse script builder in the acquisition software. Four different time-lapse types are available; constant, linear, exponential or custom. For each type, the first and last interval time can be set.

1) Constant interval time

Scan 1 · Scan 2 · Scan 3

Interval time / Experiment time

2) Linear interval time

Interval time / Experiment time

3) Exponential interval time

Interval time / Experiment time

4) Custom interval time

Interval time / Experiment time

▲ **Fig 6:** Graphical overview of the constant, linear, exponential and custom type for the script builder in Acquila. For each type, the first and last interval time can be set.

Reconstruction:

Reconstruction of time-lapse datasets differs from dynamic experiments. Both approaches will be explained in this section.

In case of a time-lapse experiment, the individual acquisition folders can be reconstructed individually or in batch. For the batch reconstruction, in a first step, a parameter set (scatter correction, beam hardening correction, phase retrieval…) will be determined on the first acquisition. In a second step, a script will be built to apply all the parameters of the first acquisition on all the other datasets. The center of rotation can still be automatically determined for each dataset individually. As a results, all datasets will be reconstructed automatically with the same reconstruction parameters for each dataset. This is needed if the user wants to perform visualization and/or analysis steps afterwards.

Another possibility is that during the acquisition of a time-lapse experiment all the information of the X-ray system will be automatically transferred to Acquila. Because Acquila is fully optimized for GPU computing, on-the-fly reconstruction becomes possible.

As a standard workflow for reconstruction of dynamic experiments, projections acquired during any full rotation of the system can be reconstructed regardless of the projection angle. This can be very important when discrete events are present inside the sample. The user can take a reconstruction block just before and after the event. In this way, there won't be any motion blurring artifacts on the images. The software has a sensor aided optimization by coupling the data of peripheral equipment (pressure stage, temperature stage…) to the reconstruction to choose the correct intervals.

In other words, the reconstruction window can be chosen depending on the dynamic process. If needed, multiple rotations can be combined to increase image statistics. In addition, temporal oversampling is possible,

where the user can define the starting point for each reconstruction block. In a standard workflow, the starting point will be just after the end of the previous block. However, it is also possible to set the starting point within the reconstruction block of the previous block. This is called temporal oversampling.

In case of the dynamic scan of the seeds, no discrete events were captured and consecutive reconstruction blocks were selected with a temporal resolution of 4 minutes. The software automatically detects the 0-360° intervals and makes a correction for the angle for the last projection. By interpolating the complete angle overshoot over the series of projections. If needed, all reconstruction blocks can be set manually as shown in figure 7.
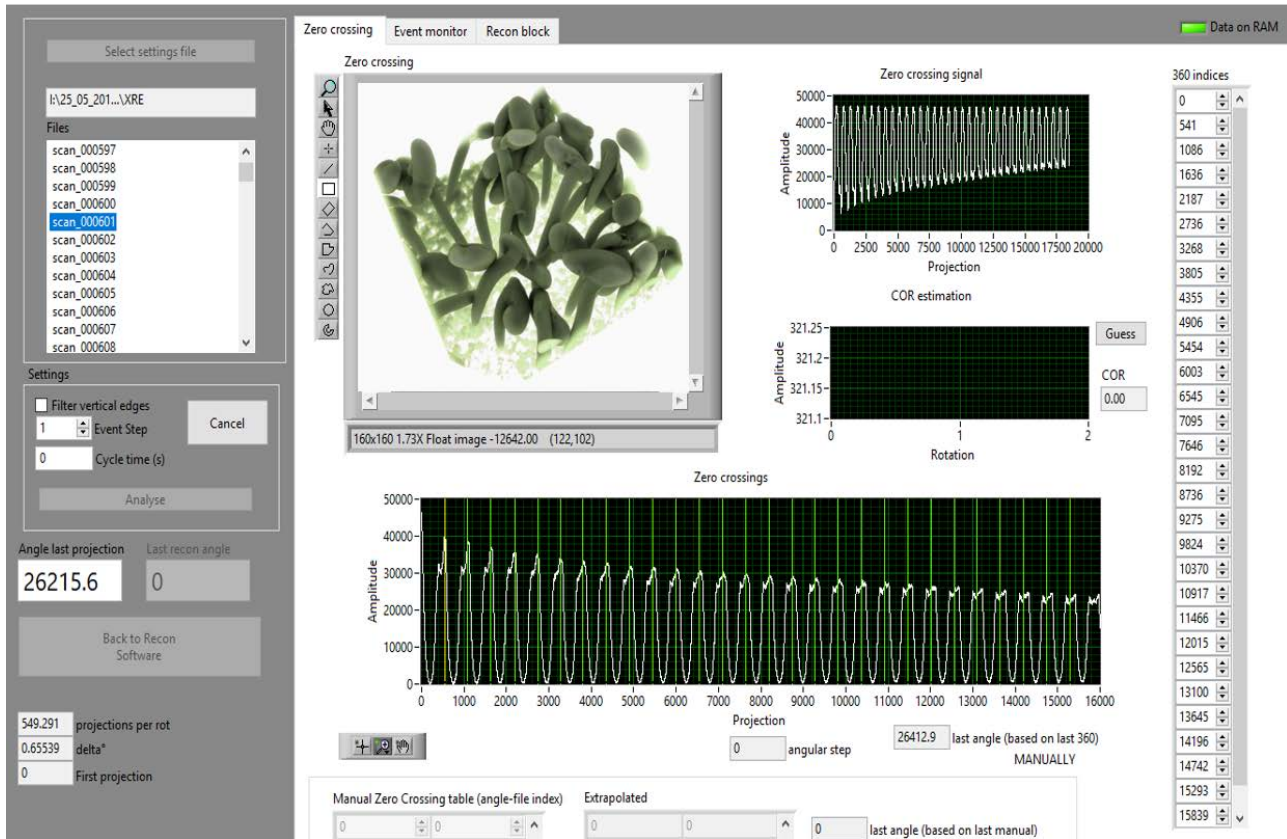
▲ **Fig 7:** Interface of the dynamic reconstruction module. The reconstruction windows can be chosen afterwards and coupled to the process. Corrections for the last angle are possible.

Results and discussion

An overview movie of both the time-lapse and dynamic experiment can be found here:

http://bit.ly/germinating_seeds

Three different time points of the time-lapse sequence can be seen in figure 8. The 3D rendering was obtained by using Drishti (open source tomography visualization software: http://sf.anu.edu.au/Vizlab/drishti/). The germination and growth process is clearly visible in the 3D reconstruction. In a first stage, the cress seeds expand and the seed coat cracks, due to the growth of the cotyledon. After the radical emerges, the cress seeds are lifted from the tissue. As can be seen on figure 8, also the cracked seed coat is lifted. In the last stage, the embryonic leaves open and give rise to the first leaves.

Visualization of three timesteps for the dynamic high resolution experiment are shown in figure 9. The dynamic experiment started 56 hours after the seeds were placed on a wet tissue, thus only visualizing the emerging radical. On the figure only 1 seed is shown. The cracking of the seed coat and the growing of the radical can be investigated. Figure 10 shows the corresponding 2D slices through the seed.

In addition, for the dynamic experiment, the datasets were analyzed by using a single threshold step. The volume of the individual seeds, including the radical, was calculated for each timestep.

The quantitative results for the seeds are shown in figure 11. Only two seeds are shown here because for the third one it was not possible to quantitatively separate it from the wet tissue. The significant increase during the first stage corresponds to the growing stages of the cotyledon. Towards the end, the growth rate slows down. As the experiment only started after 56 hours, the rapid increase in volume during the very first stages is not captured.

▲ **Fig 8:** 3D render of three different timesteps of growing seeds during the time-lapse interval. 100 acquisition (5 min scans.) were taken with a time interval of 1 hours. The total experiment amounted 130 hours.
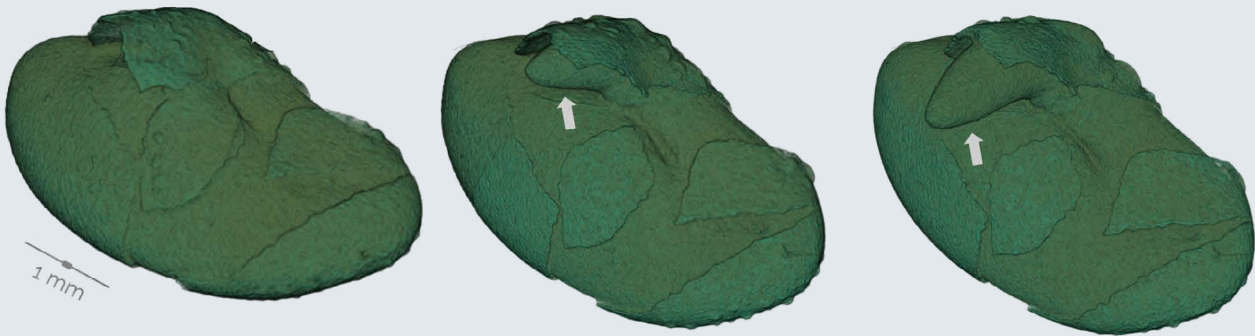
▲ **Fig 9:** Three different timesteps of the high resolution dynamic scan (9 μm, 4 min/scan) during the germination process. In total 69 reconstruction windows were selected from the continuous acquisition.
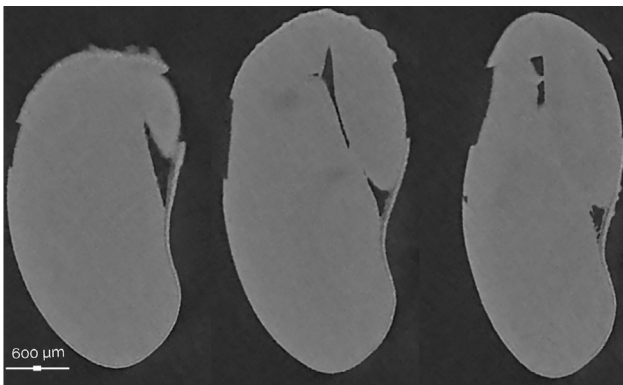
▲ **Fig 10:** Cross section through the seeds during the germination process. In total 69 reconstruction windows were selected from the continuous acquisition. The spatial resolution is 9 μm.
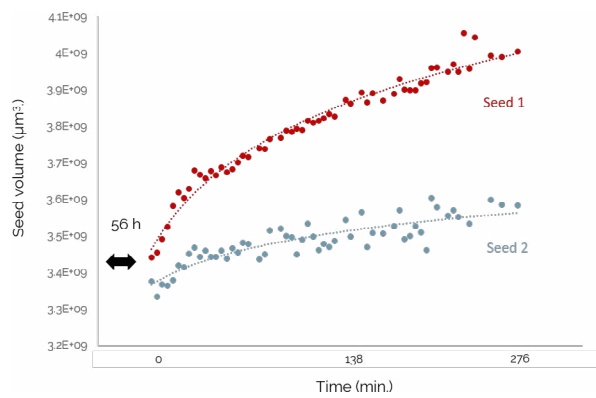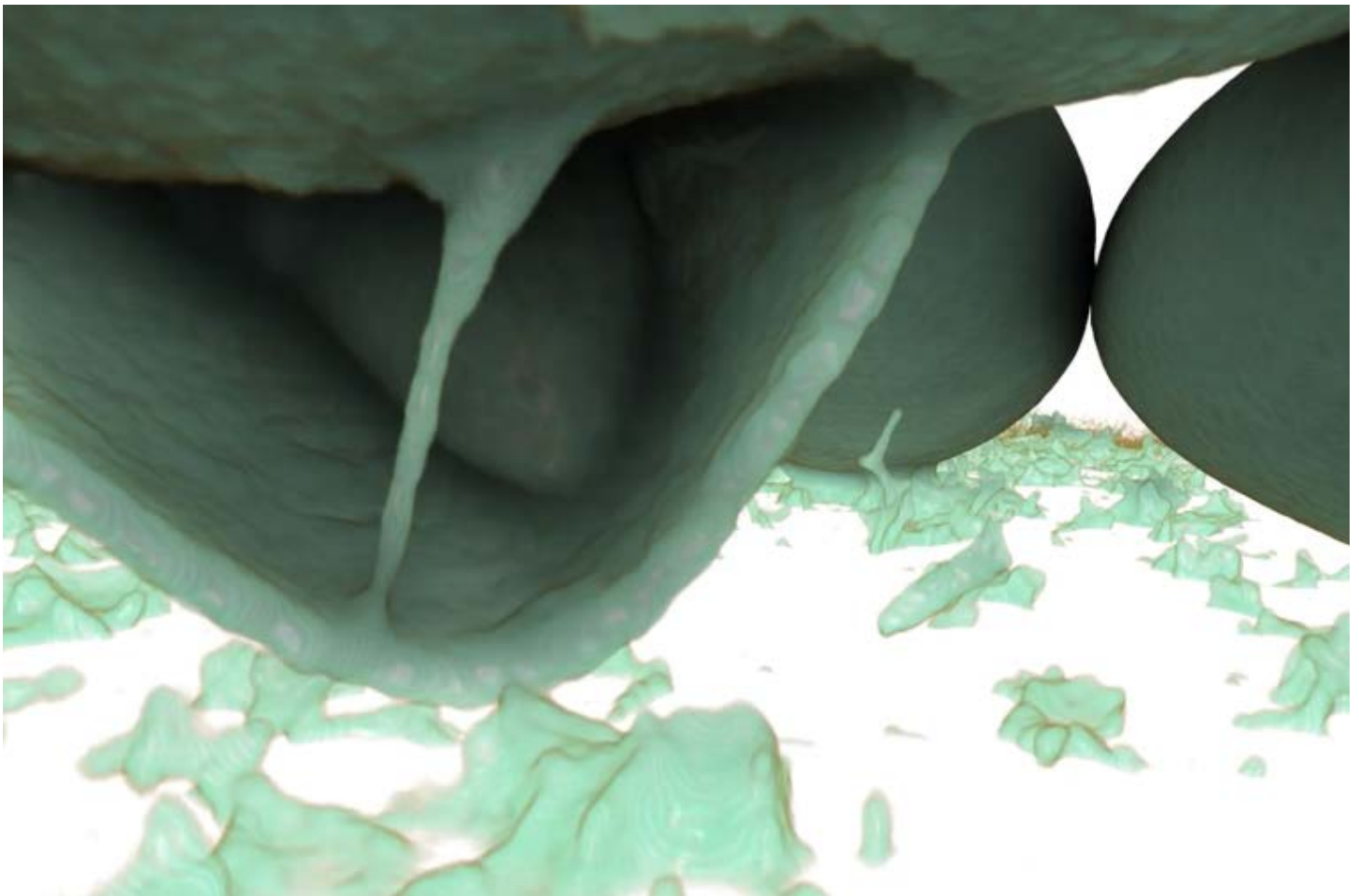
▲ **Fig 11:** Quantitative analysis of the high resolution dynamic experiment. For each time interval, the total volume of the seed (including emerging radical) was calculated. The experiment started 56 hours after the seeds were place on the wet tissue.

Conclusion

Both time-lapse and dynamic CT experiments were performed to better understand the germination processes of seeds. Different acquisition and reconstruction steps are needed for both approaches. Time-lapse experiments can be treated as individual scans and can be batch processed afterwards. Dynamic experiments on the other hand are treated as a single scan acquisition and divided in different reconstruction windows afterwards. Because of the flexibility in both hardware and software integrations of TESCAN XRE, it becomes possible to perform fast dynamic experiments in the laboratory in a user friendly way.