Fossilized but functional – Tomographic insights into nature’s most resilient actuators

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Introduction
Hygric actuation is a widespread and evolutionary very successful principle for generating motion in plants, driving – among other examples – the closing and opening movement of certain inflorescences and of pine cones. In the case of pine cone scales, this actuation principle has proved one exceptionally durable with fossilized cones of pine and related fossil species from the Middle Miocene still exhibiting considerable actuation [1]. Thus it seems safe to say that hygric actuators are the functionally most resilient actuating systems known.

The physical basis of the actuation is the difference in hygric swelling between different tissues inside the cone scale. While this has been qualitatively known for a long time, the underlying shape changes of the different tissue components inside the scale are yet not fully known. Many studies devoted to the actuation mechanism make use of invasive approaches such as sawing the cone into two halves for an optical observation of the actuation moment [2]. While this clearly brings experimental advantages by enabling the use of standard optical imaging techniques, there are serious changes in the water migration behaviour inside the pine cone as a side effect of the preparation method.

X-ray microtomography offers an interesting tool to study both the 3D microanatomy of the different tissues inside the cone scale and the shape changes of these structures occurring during hygric actuation.

Method
X-ray micro-CT was conducted on a Bruker Skyscan 1172 system using non-filtered 40 keV x-rays. Individual scales of Pinus sylvestris were studied in a dry state at ambient moisture (approximately 50%) and in a moist state after storage in a closed container with a water-soaked sponge. In order to avoid drying of the moist scales during the µCT measurement, they were placed inside closed PE tubes. For in-situ drying studies, the same type of PE tube was used with a hole inside the lid in order to allow slow evaporation of the moisture. The scale was fixed to the bottom of the tube with modelling wax in a way that assures keeping enough space for the bending movement. This fixation was done with the dry scale before conditioning in moist atmosphere. In figure 1, a sketch and a photo of the scale inside the plastic container are presented. For observation of the actuated movement, the multiple scan function of the scanner software was used. Working with the coarse (1k) camera resolution and 8 signal averages, a time series of tomographic data sets taking about 14 min to record was recorded.

A further series of experiments was conducted on a small entire cone of Pinus sp. Due to its dimensions in the range of only about 2.5 cm in diameter in the open state, this cone allows imaging of the actuated motion of the intact scales attached to the rhachis of the cone. Due to the slower actuation speed the drying of the moist-conditioned cone could be followed without a PE container around the sample in this case.
Figure 1: Top view (left) and side view (right) of the pine cone scale glued into the PE container

**Results: single scale**

In figure 2, a 3D crosshair view of the “dry” pine cone scale at about 50% r.h. is given along with the magnified view of a section taken along the scale. In figure 3, a similar crosshair view and detail section is given for the moisture-saturated scale. Due to the more complex geometry of the bent scale in “wet” condition, it is not possible to obtain a fully comparable view of the data in this case. Even with this problem not appropriately solved yet, the differences in thickness and x-ray absorption (both sets of images produced with the same false colour lookup table) are rather obvious. It is also interesting to note that even the moist scale is far from being “filled” entirely with water. Rather, there exist still many large air-filled cellular structures also in the moist state. By contrast, the sklerenchyma fibers undergo much less change in thickness and X-ray absorption.

As will be shown in the presentation, the hygric movement is relatively fast in the beginning of the drying process (even manifesting itself in motion artifacts in the first image data sets) and then levelling off during the further course of the drying process.

Natural hygric actuators such as pine cone scale may serve as an inspiration for the construction of artificial hygric actuators. Such actuators may find useful applications especially as passively switching components to prevent unwanted moisture accumulation in building parts or as sun shading devices.
Figure 2: Pine cone scale at 50 % r.h. Sample imaged with a resolution of 6.09 µm and untreated 40keV X-ray
Figure 3: Pine cone scale in fully wet stage. Sample imaged with a resolution of 6.09 µm and untreated 40keV X-ray
Results: intact cone
In figure 4, a 3D crosshair view of a small pine cone in dry state at about 50% r.h. is given. It is interesting to see how the structures at the upper side of the scales continue directly into the rachis. In figure 5, the same cone is shown directly after incubation at 100 % r.h. for 1 day. The crosshair position was chosen in a way that it is located at the center of basal side of a cone scale throughout the actuation process. Note the changes in shape, thickness and absorption contrast of the sklereid compartments at the lower sides of the scales. It is also interesting to see the extension of the sklereids, which are located only in the basal part of the scale. Furthermore, it is interesting to note that there is also notable loss of signal in the upper parts of the cone scales and in the rhachis which suggests that also in these tissues a considerable water uptake takes place during incubation at 100% r.h.

Figure 4: Intact *Pinus* sp. cone in dry state at about 50 % r.h. Sample imaged with a resolution of 26.79 µm and untreated 40keV X-ray. The image was recorded at the end of a drying series started with the cone incubated at 100 % r.h. for a day. The image of the moist cone is shown in figure 5
Results: other hygric actuator geometries

Natural hygric actuator systems are not limited to a primarily bending actuation as found in pine cones. The petals of some flowers such as *Carlina acaulis* exhibit a complex sequence of several bending actuations in different regions of the petal which are occurring at different moisture levels. In some conifers such as *Cupressus sempervirens*, the cone scales show a different form than pine cone scales and are arranged in a different way around the cone rhachis (see figure 6) leading to a more complex 3D hygroscopically actuated motion pattern.
Figure 6: 3D rendering of a *Cupressus sempervirens* cone in open state at ambient conditions. In the cutout region, the sclerenchyma fibres are visible. In contrast to the linear arrangement in *Pinus* cone scales, they are arranged in a conical way under the umbo which is retracted back during hygric actuation.

**Discussion**
The images demonstrate how X-ray µCT provides deeper insights into the functional anatomy of the pine cone scales and cones of other conifers during hygric actuation. By imaging an intact cone, the role of the anchoring tissue during the actuation process can be studied as well. Further and more quantitative insights into the actuation process seem retrievable from the images. However, due to the lack of an appropriate elastic registration tool, such analyses have not yet been conducted.

**References:**
1. S. Poppinga et al., “Hygroscopic motions of fossil conifer cones” · Scientific Reports 7:40302, DOI 10.1038/srep40302, 2017