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Review article

Looking deep into nature: A review of micro-computed tomography in biomimicry

Anton du Plessis [a,b,*], Chris Broeckhoven [c]

[a] Physics Department, Stellenbosch University, Stellenbosch 7602, South Africa
[b] CT Scanner Facility, Stellenbosch University, Stellenbosch 7602, South Africa
[c] Laboratory of Functional Morphology, Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

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A B S T R A C T

Albert Einstein once said "look deep into nature, and then you will understand everything better". *Looking deep into nature* has in the last few years become much more achievable through the use of high-resolution X-ray micro-computed tomography (microCT). The non-destructive nature of microCT, combined with three-dimensional visualization and analysis, allows for the most complete internal and external "view" of natural materials and structures at both macro- and micro-scale. This capability brings with it the possibility to learn from nature at an unprecedented level of detail in full three dimensions, allowing us to improve our current understanding of structures, learn from them and apply them to solve engineering problems. The use of microCT in the fields of biomimicry, biomimetic engineering and bioinspiration is growing rapidly and holds great promise. MicroCT images and three-dimensional data can be used as generic bio-inspiration, or may be interpreted as detailed blueprints for specific engineering applications, i.e., reverse-engineering nature. In this review, we show how microCT has been used in bioinspiration and biomimetic studies to date, including investigations of multifunctional structures, hierarchical structures and the growing use of additive manufacturing and mechanical testing of 3D printed models in combination with microCT. The latest microCT capabilities and developments which might support biomimetic studies are described and the unique synergy between microCT and biomimicry is demonstrated.

Statement of significance

This review highlights the growing use of X-ray micro computed tomography in biomimetic research. We feel the timing of this paper is excellent as there is a significant growth and interest in biomimetic research, also coupled with additive manufacturing, but still no review of the use of microCT in this field. The use of microCT for structural biomimetic and biomaterials research has huge potential but is still under-utilized, partly due to lack of knowledge of the capabilities and how it can be used in this field. We hope this review fills this gap and fuels further advances in this field using microCT.

Contents

* Corresponding author at: Physics Department, Stellenbosch University, Stellenbosch 7602, South Africa.
E-mail address: anton2@sun.ac.za (A. du Plessis).

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1. Introduction

Biomimicry involves the process of learning from and emulating nature [1–3]. One the major aims of biomimicry is to make use of biological inspiration or input to develop new improved materials and find new solutions to engineering challenges [4]. The field of biological materials science – the study of the mechanical behavior of biological materials – has grown steadily in recent years, in part due to a major interest in the biomimetic potential of these materials [5,6].

Natural structures are the result of millions of years of evolution and are generally thought to be optimized to fulfil specific functions. Consequently, they are particularly useful for designing novel artificial materials. Convergent evolution of similarly-shaped structures has occurred in a variety of phylogenetically-unrelated organisms. An example of this is the development of specialized animal weapons, discussed in terms of lessons for biomimetic materials designs in [7]. Another example is the basic structural components which are present in various biological materials, and which have been broadly categorized into eight natural structural elements for biomimetic design [8]: fibrous, helical, gradient, layered, tubular, cellular, suture and overlapping structures. Of particular interest is not only the structure itself, but also the combination of structural elements, as well as the gradients between structures which can combine to form materials with far superior properties compared to the equivalent individual parts with no gradient or with only one of the two structures [9]. In the same context, structural elements which repeat themselves over various length scales in the same material occur often in nature, alsoproviding superior properties. These hierarchical structures are discussed more in [10]. All in all, an improved understanding of the design elements and their interrelationships on various levels may provide powerful new bio-inspired design rules for creating engineering materials with exceptional properties. Pivotal for this improved understanding is an excellent and accurate visualization and measurement of the dimensions and properties of the biomaterials investigated, to accurately quantify the natural elements in three dimensions.

For visualization and detailed analysis of biomaterials, X-ray micro computed tomography (microCT) has a particularly powerful role to play. The non-destructive nature of the technique allows visualization of virtual slice images of internal details irrespective of the properties and without potential damage to the biological material investigated (e.g., brittle materials, wet or fragile materials which may change their shape when sectioned physically, etc.). While microCT is sometimes used to provide such a virtual slice image or even a striking 3D image of a biological structure in biomimetic studies, it is most often used solely for imaging and not for more detailed analysis of the three-dimensional structure using advanced measurements. In most biomimetic studies (regardless of whether microCT is used or not), bioinspiration is often taken only broadly from images or observations (often optical or electron microscope images). A good example of this is the recent review of the use of additive manufacturing technology in biomimetic engineering studies, which only includes two examples of the use of microCT [11]. This is however starting to change as some recent studies have started to make use of the full capabilities of microCT for reverse engineering, hence true reverse-engineering of natural structures using microCT has now become possible. Two recent examples are the protocol outlined for reverse engineering a sea urchin mouthpiece (known as the Aristotle's lantern) as a unique gripping device [12] and a similar workflow investigating the solid shell (cortical bone) and porous core (trabecular bone core) combination of glyptodont osteoderms (i.e., bony plates embedded in the skin), which could be applied to impact-resistantmaterials [13]. In this context, a recent review also discusses the usefulness of 3D printing biological models, often obtained from microCT, for mechanical testing [14].

X-ray microCT has grown in recent years from a qualitative imaging tool to a quantitative analytical tool with great benefit to a variety of fields as has been reviewed for materials sciences [15], biological sciences [16], industrial applications [17], and additive manufacturing [18]. While it is often used in biological materials science as an imaging tool, its use is still growing and developing especially in biological studies [16,19–21].

The method is not only expanding in its wider use and availability, but some new visualization and analysis methods are being developed which are of particular interest to the field of biomimicry and which are discussed in this review in more detail – specifically advanced 3D morphological measurements (e.g., wall or strut thickness, pore size), and image-based simulations (e.g., permeability simulation or structural mechanics simulation of microCT data of real objects, in contrast to simulation on idealized models). These new capabilities have developed in recent years and are still being developed due to improvements in computing hardware and 3D image analysis software tools, allowing the tech-

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nique of X-ray microCT to provide more than simply an image – it now also provides quantitative analysis options to further advance the field of biological materials science and biomimetic engineering, to further drive innovation and find solutions to engineering challenges using inspiration from nature. Thus, the aim of this review is to highlight how microCT has been used in biomimetic studies to date, and to present the latest capabilities and opportunities in this field.

2. X-ray microCT method & technology

The microCT scanning technique is schematically illustrated in Fig. 1 using the example of a single snake fang; the schematic is a modified version taken from [22]. X-ray microCT is referred to by many names including X-ray microtomography, X-ray computed tomography (XCT), CT scanning and X-ray microscopy. Generally speaking, these all refer to the same technique. The method makes use of an X-ray source, a rotating sample and digital detector. The source is a microfocus X-ray source producing a cone beam of X-rays which are sent through and around an object of interest, creating a shadow image on an X-ray detector. This digital detector acquires 2D X-ray images (projection images) at each step position during a full rotation of the sample around an axis perpendicular to the direction of the X-ray beam. The acquired images are reconstructed using a filtered backprojection algorithm [23] to create a 3D data set comprising of volumetric pixels – voxels – where the brightness is related to the density and atomic mass of the material the voxel represents [24].

The microCT scanning process typically involves sample preparation by placing the sample in a low-density container attached to the rotation hardware of the microCT system. Generally speaking, no further sample preparation is required, but it is important that the sample remains rigid for the entire scan duration which can range from minutes to hours (typically one hour is required for a high-quality scan). Scan parameters need optimization for each sample type, with lower voltages normally being selected for biological materials to enhance contrast. Denser objects require higher voltage for more penetration capability, but this reduces the contrast between materials with similar densities. Acquiring longer and more images during a rotation enhances the quality of the obtained result, and the X-ray current can be increased to enhance the signal to noise ratio. Once data is obtained, the reconstruction takes place with some system-specific parameters in the process. Obtained data is then analyzed and visualized in dedicated software, which allows segmentation of the materials in the slice images, and consequently, 3D visualization and further processing. The general considerations for scanning and segmentation of biological sample data are detailed in [16,25].

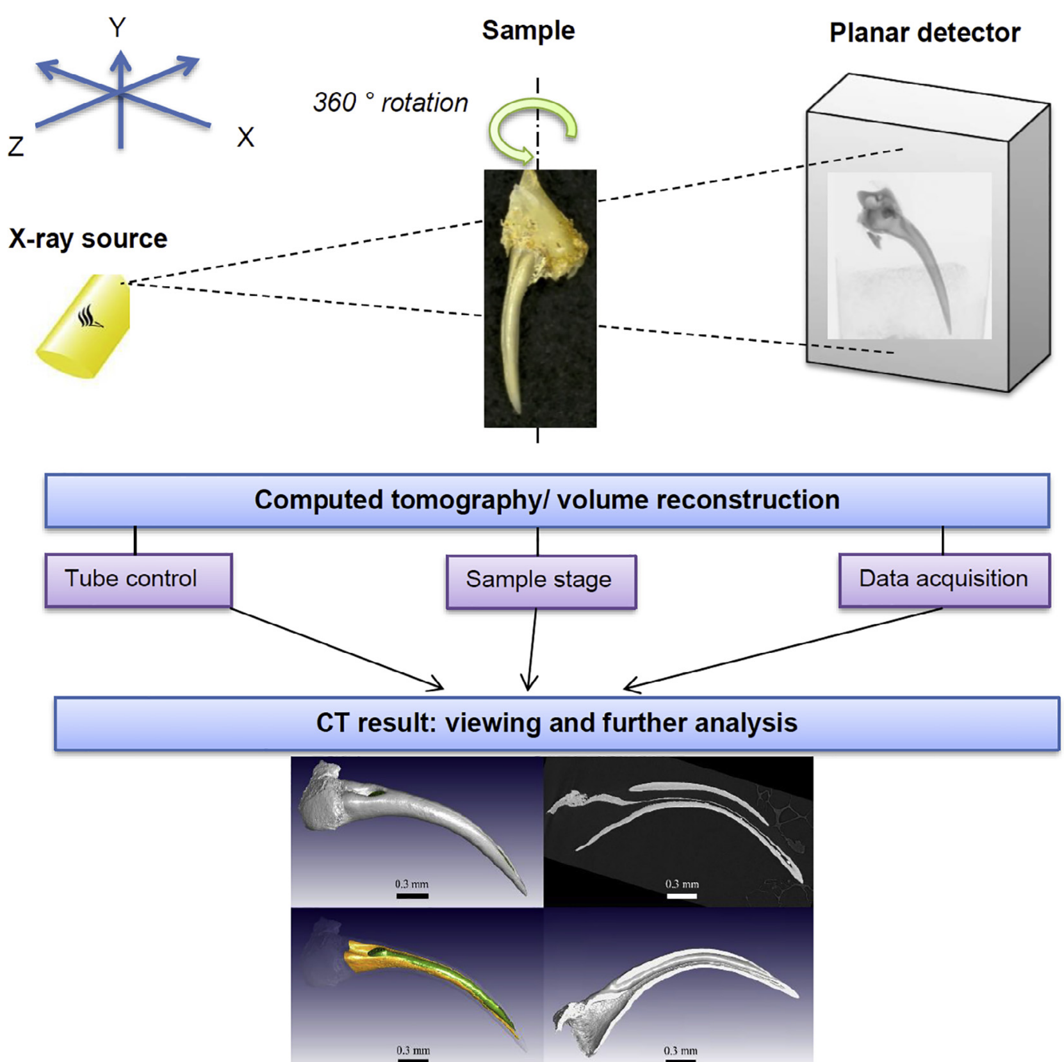

Fig. 1. Schematic of the microCT scanning process. A sample – in this case a snake fang – is slowly rotated acquiring X-ray projection images, and these are reconstructed to generate a 3D data set representing the sample, which can then be viewed and analyzed in virtual slices or 3D images.

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It is important to note that microCT scanning is not a simple "in-out" process and may be regarded as an art owing to the fact that, not only is the system used important to acquire high quality data, but the procedures used are equally important – there are hundreds of ways of scanning a sample with numerous parameters that can be varied and options that can be selected depending on the size, complexity and material type among other factors. Various types of microCT systems are available, ranging from small desktop systems for smaller samples up to large cabinets and even larger room-sized systems. The size range of microCT systems affects not only the size of sample that can be scanned but also the image quality that can be obtained, the density and material types that can be scanned successfully, and the quality of the obtained scans even with the same resolution. A comparison of microCT systems is not within the scope of this review but it is important to know that the selection of a suitable system for the object of interest is necessary. It is also important to understand that microCT provides far superior resolution compared to medical CT, but may be orders of magnitude slower due to the lower brightness source used [26,27]. The typical medical CT resolution is approximately 0.5–1 mm, while microCT extends from below 0.25 mm down to less than 1 μm.

The optimization and selection of scan parameters is the next factor affecting image quality and this may be learned through experience, through emulating prior work published on similar samples, or through use of published guidelines such as in [16,28]. Image quality is therefore affected by a combination of system capabilities and operator capabilities and experience. A quantitative measure of image quality in microCT slice images is possible through the measurement of the grey values in selected areas in the object of interest and the background, as demonstrated for a range of settings in [29]. Another important aspect is the image sharpness which may also deteriorate for a number of reasons.

For more information on the microCT scanning methodology, current systems and capabilities and the use of synchrotron tomography, the reader is referred to [18]. For more information on possible artefacts, reconstruction options and scan parameters, please see more details in [16]. Once images are obtained, 3D image analysis is possible and allows visualization and advanced dimensional and volumetric measurements. Various open source and commercial software packages are available for this purpose with varying levels of user interaction and capabilities. All examples in this paper were generated with Volume Graphics VGSTUDIO MAX.

3. Overview of microCT in biomimetic research

In this section a summary of the literature is provided where microCT was used in biomimetic research. The studies are categorized to provide an overview of the types of biological materials that have been studied using microCT, often for the purpose of biomimetic inspiration. Various other biological structures have been studied by microCT for morphological descriptions, but only those studies with a biomimetic focus are included here.

3.1. Plants

Plants have been of great interest to biomimetic studies, especially those focusing on leaf surface topography and material systems (reviewed in [30]). Although SEM imaging remains the leading method for visualizing plant structures due to their submicrometer feature sizes, a number of recent studies that employ microCT have added significantly to our understanding of plant structures and consequently opened the doors for biomimetic designs. For example, by using finite element modelling based on microCT imaging and 3D optical scanning, significant insight has been gained into branched plant stems, which, in turn, could serve as inspiration for biomimetic improvements of joints in engineering parts [31]. Gludovatz et al. [32] studied the multiscale structure of coconut shells using a combination of microCT and mechanical tests. The authors found that older shells are stronger than young shells, presumably due to the occurrence of densification as the shell ages. By making use of microCT and SEM imaging, a similar study on the fruit of the Cocoyol palm tree revealed a functionally graded material with distinctive layering [33]. In bamboo, the axial compressive strength has been attributed to the arrangement of parenchyma cells, as revealed by finite element-based modelling applied to microCT scans of parenchyma and sclerenchyma tissues [34]. Assessing the mechanical performance based on micro-CT data, or 3D-printed structures [35], might advance the development of plant-based bionic designs [34]. Lastly, the roots of the mangrove plant were studied for their ability to filter and desalinate salt water, and the microstructure was visualized with microCT [36]. The latter study could be applied to biomimetic water filtration systems.

3.2. Invertebrates

Invertebrates are particularly interesting model organisms for biomimetics (outlined in [37]), because of their extensive diversity, and many of them have been the topic of biomimetic studies using microCT. In a recent study by Petersen et al. [38], a variety of techniques, including microCT, were used to investigate the surface adhesion of barnacles on different structured surfaces. The study demonstrates that the mushroom-shaped surface topography of the barnacle does not allow good contact for the adhesion (and shows less calcification compared to a smooth surface). The results could be used to develop a simple method to minimize barnacle adhesion in marine applications [38].

Invertebrate exoskeletons have been widely studied using microCT. For example, the complex interactions between neighboring plates in the shell of the barnacle could only be identified by making use of non-destructive 3D visualization methods [39]. Similarly, the exoskeleton of the heart urchin – a strong, yet highly porous structure – was analyzed in detail by Müter et al. [40]. Analysis of strut thickness and spacing of this structure, measured using microCT, revealed that very high porosity can be achieved without compromising its strength by varying the spacing of the struts [40]. In contrast to sea urchins, the exoskeletons of crustaceans generally have layered chitin fibres, in a helical layup. This bioinspiration was used in a study of carbon fibre – epoxy laminates, with varying helical layup and mechanical testing, where microCT was employed to image damage and cracks observed in sample composites [41]. The highly porous structure of the cuttlebone, the buoyancy device of the cuttlefish, has been a topic of investigation for bio-inspiration due to its relatively high strength, very low density and good buoyancy properties – potentially useful properties for bone tissue engineering [42]. Fig. 2 shows a close-up 3D image of the internal structure of a cuttlebone sample, where the field of view shown is roughly 0.5 mm. This material was first imaged by high resolution microCT in [43].

Besides protective structures, insect weaponry, such as the mandibles of stag beetles have been analyzed by microCT in the past [44–46]. More specifically, a biomechanical investigation of the stresses in the mandibles during fights, the cost of the mandible mass relative to its flight capability and the description of mechanoreceptors on the mandibles have received considerable attention over the past few years. Beetles have also been the topic of inspiration for lightweight building designs, starting from microCT images as described in [47].

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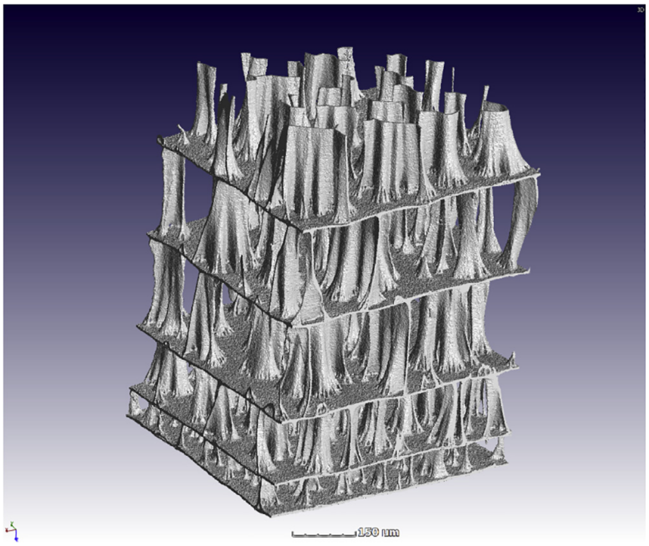

Fig. 2. Structure of cuttlefish bone in 3D, field of view here is roughly 0.5 mm. This structure is extremely lightweight while maintaining good strength and structural integrity despite the pressures exerted on it during deep dives of the cuttlefish.

The dragonfly wing hinge system was investigated using a combination of imaging and simulation methods including microCT imaging [48]. Dragonfly wings have been the topic of numerous investigations for biomimetic insights into flight systems, see for example [49]. In addition, a biological screw and nut hinge system was found in the legs of weevil beetles using synchrotron CT [50]. The morphology was described and it was speculated that this type of hinge system holds advantages for mobility during feeding, which might have resulted in the evolution of this system.

The visual fields of bees have been studied using microCT and optical modelling [51], providing insight into the visual information used for navigation in a dense forest, which can be useful for biomimetic autonomous flight systems.

3.3. Fish

Fish have been the topic of a number of biomimetic studies, particularly focused on scalation, because of their protective role as well as their ability to minimize drag in water. Alligator gar fish scales were analyzed by synchrotron tomography showing the internal tubules and their orientation relative to the crack path during fracture toughness experiments (notched three-point bending) [52]. It was found that the tubules act as crack arrestors, slowing the failure process in cyclic tests. The use of fish as a bioinspiration model system is discussed in [53]. The dermal armor of the three-spine stickleback was analyzed in detail by microCT and 3D printing, showing different armor plate morphologies, their interlocking mechanisms and thickness distributions and their porous nature, indicating its role in limiting damage from predator bites [54]. The armor of the boxfish has been a topic of investigation using microCT to visualize the shape and distribution of armor units across the fish, indicating 78% are hexagonal in shape and having interlocking sutures connecting them [55].The bones of the seahorse were analyzed by microCT and compression testing, showing very high elastic deformation capability and plastic deformation serving to prevent fracture, for example when being subjected to bite forces from predators [56]. In a recent study, microCT was used to 3D print dermal armour units (amongst others from fish) and these were used to analyze the flexural properties of combinations of armour units [57]. Much recent work has been done in 3D printing of dermal armors of various species as summarized in [58].

Biomimetic surfaces optimized for minimizing drag in water were studied based on the structure of shark skin, using a combination of microCT, 3D printing flexible skin with hard denticles and testing the hydrodynamic properties (drag) for various spacings of denticles, thereby optimizing a biomimetic shark skin [59,60]. A study which used microCT for analyzing the internal details of the Longnose skate was reported in [61]. The study is highly relevant to biomimetic designs for flexible yet strong materials for autonomous underwater vehicles.

3.4. Reptiles

Reptiles have received considerably less attention from the field of biomimetics, despite the extensive variation in structures present in this group. Recent work by Broeckhoven & du Plessis [62] used microCT to investigate the detailed internal and external morphology of snake fangs within a phylogenetic framework. The authors described three distinct morphologies and argue that snake fangs might be biomechanically optimized for biting and piercing prey [62]. This work was extended with advanced morphological analyses and made use of direct microCT-based load simulation [63]. This capability is relatively new and the methodology is outlined in detail in the supplementary material associated with this paper. Since fangs are optimized for injection and envenomation, the data might be particularly useful for future bioinspiration of surgical needles.

Like insects and fish, various reptiles possess protective structures, including carapaces and osteoderms. In a study of the leatherback sea turtle shell, microCT was used to visualize and describe the three-dimensional structure of the interlocking sutures [64]. In addition, the structure of turtle carapaces has been described using microCT in earlier work [65]. The protective nature of lizard dermal armor has recently been described, as well as the potential role that these structures might play during thermoregulation [66]. Using a newly developed protocol, these authors also acquired microCT images of live lizards in a standard microCT instrument and this allowed the visualization of the lizard in defence mode [67]. Fig. 3 shows a live lizard microCT data set from this work, with load simulation applied to its osteoderms.

3.5. Birds

Birds are unique in their flight ability, therefore lightweight bones and aerodynamic properties are sources of inspiration from birds with numerous possible applications. Due to the extreme low weight of bird bones, which are expected to be critical to their flight capacity, various aspects of bird bones and feathers have been studied. MicroCT has provided insightful information on the reinforcements in bird wing bones, as well as feather morphology [68,69].

Besides flight, the beaks of birds have been the focus of biomimetic and/or bioinspired studies. The mechanical properties of the Toucan beak were studied by microCT and finite element modelling [70], due to its strength and low mass. Toucan and hornbill beaks were compared using a variety of techniques including microCT for studying the internal morphology of the beak [71].

Another study of the shock absorbing capability of the woodpecker was initiated in 2010 showing a biomimetic design outperforming a traditional resin based design for shock absorption in micro-devices [72]. More recent work on this topic used microCT to compare the bone morphology of chickens and woodpeckers indicating areas of higher density and thickness, and specific differences that can be related to the shock absorbing capability of the woodpecker [73]. More detailed structural analysis of the bone morphology of the woodpecker using microCT is reported in [74].

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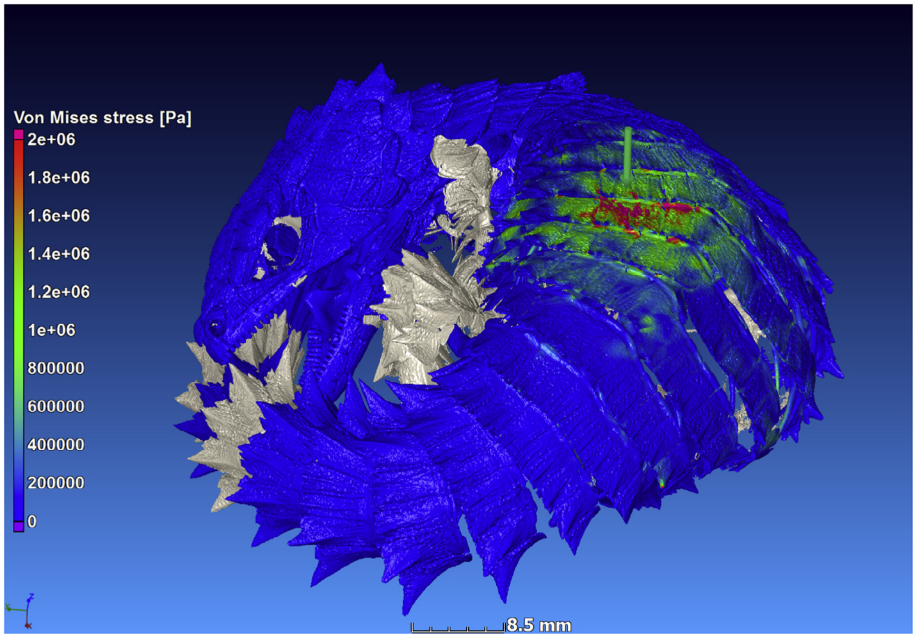

Fig. 3. Visualisation of the osteoderms of the armadillo lizard, with load simulation applied to microCT data. The simulation is applied to the osteoderms and bone modelled as homogeneous elastic material, with a circular load applied at the arrow. The stress distribution can be used to understand the interrelationship among adjacent osteoderms for "bite" loads applied at different places and in this case a live lizard in rolled-up defence mode could be analyzed.

3.6. Mammals

Mammals are sources of bioinspiration for various applications including protective materials, strong materials from animal weapons (horns, spines) and a large amount of work is done on biomimetic bone tissue engineering for improved healing as described below.

A review of the structure of keratin-based materials across many species, mainly defensive in nature, is reported in [75]. Energy absorbing natural materials (also mainly from defensive natural materials) and design strategies based on these are also reviewed in [76]. MicroCT has been used to visualize the internal structure of osteoderms as early as 2009, showing the interlocking suture structures in 3D [77]. Some recent work investigated the impact protection capabilities of bio-inspired ceramic armour plates based on the geometry of fish scales and the osteoderms of armadillos [78]. The armadillo osteoderm has been previously studied for its protective role [79]. Despite the significant interest in protective scales and osteoderms, most studies did not make use of microCT for detailed internal morphological analysis. The protective role of the glyptodont body armour was analyzed in [13], using microCT scans, simulations and a reverse engineering approach with 3D printing of models with design variations and mechanical testing. The glyptodont (an extinct mammal) is an interesting model system as its armour presumably evolved to withstand strong tail club blows from conspecifics. Pangolin scales were studied by microCT for their protective properties, in particular the crack paths across the scales were imaged after mechanical tests [80]. Furthermore, gradient structures have been studied for their enhanced protective role, especially improving the performance at an interface between different materials, see for example [81] and [9]. Hedgehog spines were analyzed by microCT and mechanical testing recently [82], showing an interesting structure with a dual structured foam core, presumably increasing flexural strength while minimizing mass. Previous work on specialized porcupine quills with barbs compared to similar quills without such barbs, showed that the barbs enhance tissue penetration at the same force, using microCT for quantification of tissue penetration depth non-destructively [83].

Besides body armour, various mammal species have evolved extensive weaponry [84]. The horns of bighorn sheep were analyzed by high resolution microCT, showing the 3D distribution and orientation of tubules [85]. These tubules play an important role in impact energy absorption, and deformation mechanisms were analyzed. Another study of bighorn sheep using microCT made use of FEM simulations to better understand the role of the horn length and foam core on preventing damage during impact [86].

Seal whiskers have been studied by microCT for detailed analysis of their surface morphologies, which are known to be optimized to reduce water drag and might serve as bioinspiration for optimized flow designs [87].

3.6.1. Humans

Bone tissue engineering is probably the area attracting the greatest interest for biomimicry; the aim is to create scaffolds conducive for new bone growth, especially in bone implants. Attempts at mimicking trabecular bone structure have been widespread, and the topic is reviewed in a number of recent works [88–90] related to additive manufacturing of scaffolds (laser powder bed fusion in particular). However, the design and density of the lattice structure affects the strut and pore sizes which affect its functionality, ie. mechanical and other properties, as has been investigated numerically in [91]. Recent work in strategic biomimetic approaches to lattice design was performed by using information from the biological structure of interest only [92].

4. Advanced microCT capabilities for biomimicry

MicroCT has been used for a number of years in a variety of biomaterials and biomimetic studies, but it has been used mainly to visualize the inner structure of materials, rather than make accurate dimensional measurements or advanced 3D analysis. This is one major gap which is now becoming an opportunity for future researchers to take advantage of, i.e. to learn from nature on a microscale in full three dimensions. Associated with this is the possibility to make image-based simulations on natural structures directly, which has been limited in the past. Furthermore, microCT data can be transferred almost directly to 3D printing for mechanical and other tests, to directly examine natural reverse-engineered structures. Although advanced microCT analysis methods are used by some, many of these methods are still relatively unknown to most biomimetic engineering researchers. In addition, new methods are constantly being developed. In this section we describe the most useful advanced capabilities which will support biomimetic research efforts in future studies, using examples from our laboratory [93].

4.1. Data handling, de-noising and visualization

An important aspect of microCT is the size of the data file, which typically ranges between 1 and 20 Gb for a single scan volume. In the past, this has limited the capabilities of the microCT technique because it was challenging to generate images of sufficiently high quality. Large workstations with significant amounts of memory, relatively fast processors and good graphics cards are required to handle 3D image rendering and further advanced analyses. Nowadays, these workstations are becoming more accessible and affordable. Irrespective of the computing power available, there are some general guidelines for efficiently handling large data files. Firstly, de-noising of images is good practice and eases the subsequent segmentation, especially when automated thresholding methods are used. In addition, these image de-noising filters also allow simpler 3D image rendering. Useful de-noising methods include the adaptive Gauss and non-local means filters. This latter filter works extremely well to keep the edges sharp while smoothing the internal and external regions, with the disadvantage of being relatively slow. Secondly, visualization in three dimensions may be achieved using a variety of rendering methods. The first requirement is image segmentation, i.e., separating the area to be visualized in all 2D images representing the volume, prior to imaging it in three dimensions. The 3D rendering may be improved by numerous methods, including smoothing of regions of interest and removing small unwanted areas, ensuring a "cleaner" image. Often 3D images may seem cluttered and difficult to visualize the context of the feature of interest – in this case cropping the 3D image helps to remove unwanted complexity while still retaining the 3D nature of the image. Finally, 2D images themselves can be used in a variety of ways besides the usual contrasting methods. A method not widely known yet is the use of a "thick slab mode", which allows a single slice image to represent a large number of slices and effectively only show the brightest or least bright voxels of all slices along a single viewing direction in one image. This can be useful to demonstrate alignment of features along a single direction. Furthermore, an additional 2D feature that may be useful in biological analyses include viewing non-flat surfaces in a single flattened slice image, using an "unroll" function. This is demonstrated in Fig. 4 on the curved biting surface of the teeth of the Eagle ray, *Myliobatis aquila*, unrolled to view the curved surface in a flat slice image.

4.2. Advanced dimensional analysis, porosity & foam structure

As outlined in the summary of biomimetic works to date, many biological structures exhibit unique porous structures of varying strut thickness, density and gradients. Analyzing these in more detail for the purpose of biomimicry is one of the hidden gems of microCT analyses, as the tools have been perfected for analyzing bone structure, metal foams and other engineering structures in the past. For example, using these functions for detailed analysis of the internal structure of glyptodont osteoderms (as shown in Fig. 5), allowed for the creation of reverse-engineered models with engineered lattice struts with the same strut thickness [13]. This study was performed to investigate the energy absorption properties of this unique structure, which evolved presumably to withstand very high impact tail-club blows from conspecifics during fights.

Other dimensional analysis options which are of interest to biomimetic research include material volume fraction and other morphological indicators. Measurement of strut thickness using a sphere-growing algorithm provides the largest sphere diameter fitting into every region inside the structure of interest [95] and is a very useful and simple way of visualizing the thickness distribution of any structure, not only trabecular structure, either for material or for background analysis (e.g., connected pore spaces). Advanced porosity analysis is possible using a variety of algorithms for color-coding pore spaces which are disconnected from one another (e.g., porosity analysis). Foam structure analysis is a specialized tool for measuring pores which are connected to one another, effectively splitting the pores and providing information on the pore sizes and struts between them. Similar methods of

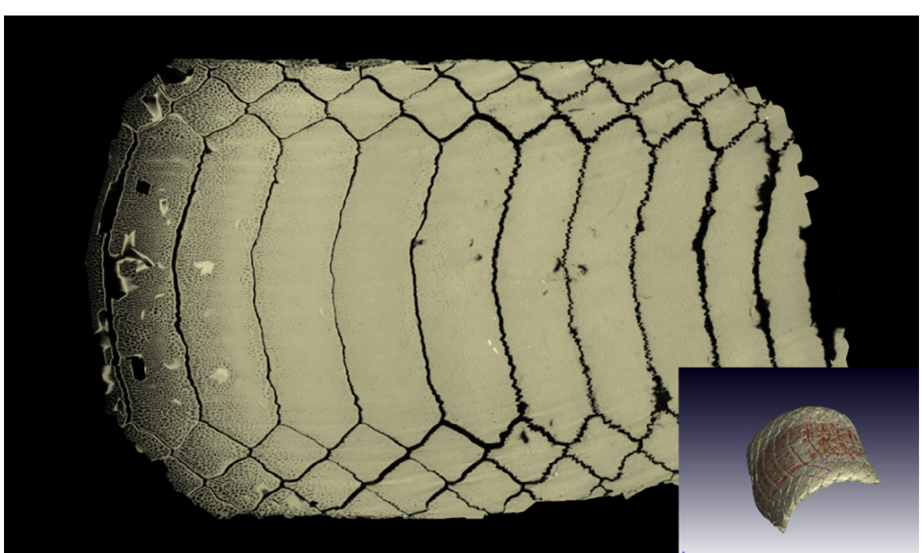

Fig. 4. Unroll mode allows visualization of the curved biting surface of the teeth of the eagle ray. The damaged part on the left is where the mollusks are crushed and constant new crushing surface is grown from the right to replace the damaged surface. Sample provided by Jannes Landschoff and Craig Foster from the Sea-Change project [94].

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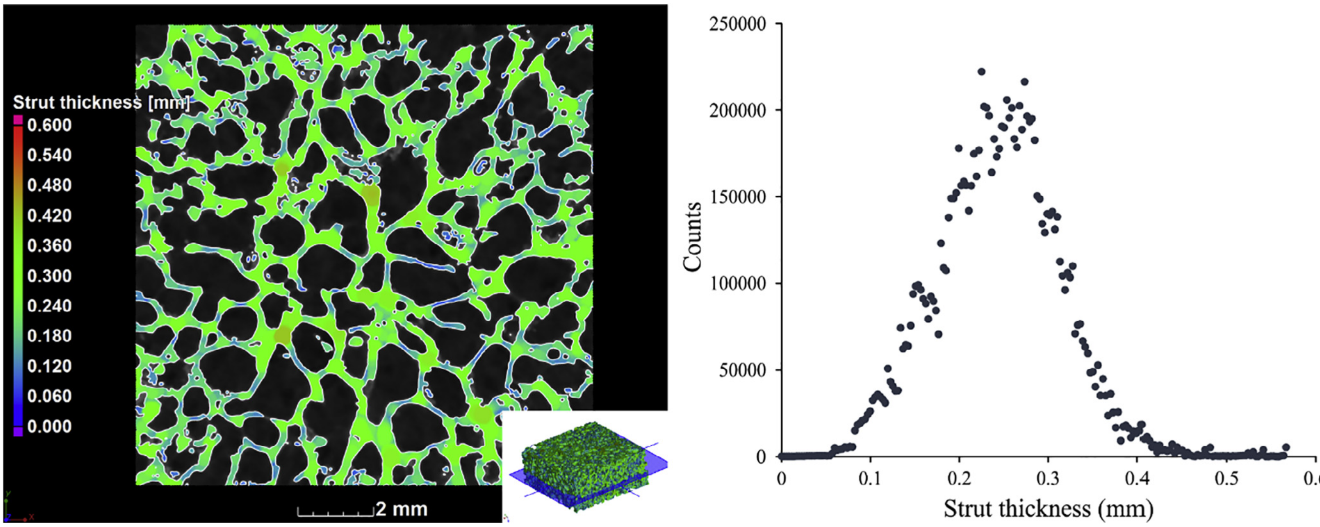

Fig. 5. Strut thickness in a glyptodont osteoderm, taken from [13]. Shown here is a selected cube from the inside of a single glyptodont osteoderm, with strut thickness analysis according to the maximal spheres method [95].

splitting connected pores in 3D are possible making use of algorithms such as the watershed segmentation method. Another possibility for connected pore spaces, especially for branching connected systems is skeletonization. This uses an algorithm to create a central point at every location along the branching pore space, making a simplified 3D representation of the skeleton network possible and provides quantitative information such as number of nodes (branch splittings) and segment lengths (branch lengths), amongst others.

4.3. Surface roughness & coating thickness

A recent functionality which has been developed due to engineering requirements in other fields, is the imaging and quantitative assessment of surface roughness or topography, as demonstrated in [18,96]. This is possible on biological samples as shown in Fig. 6 allowing a clear indication of maximum positive and negative deviations from a mean value (height map), and quantitative evaluation of roughness from data values extracted from this analysis. Similarly, a useful tool for biomimetic research is the analysis of a coating or layer thickness across a surface, as demonstrated for the epidermal layer covering an osteoderm in the armadillo lizard (Fig. 7).

4.4. Image-based simulations

One of the great capabilities of microCT data, which is particularly relevant to biomechanics and biological materials science, is the possibility to use the three-dimensional image data for simulations to better understand the material properties compared to idealized models. Two simulation methods are of particular interest and are discussed here: structural mechanics and permeability.

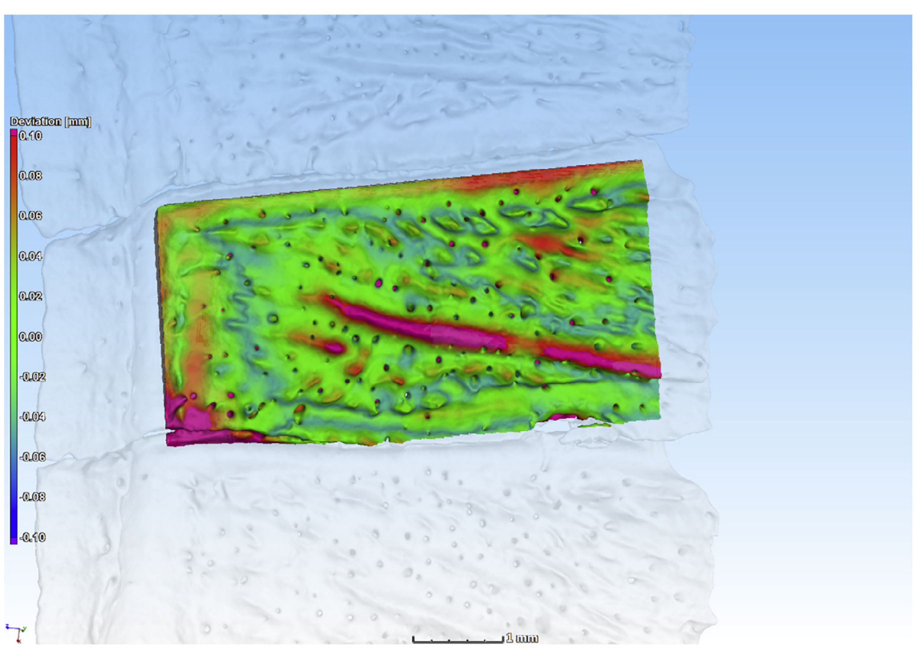

Fig. 6. Surface roughness of a single osteoderm of an armadillo lizard, highlighting ridges and troughs. Color-coding is based on deviations of the surface height relative to a mean planar surface area.

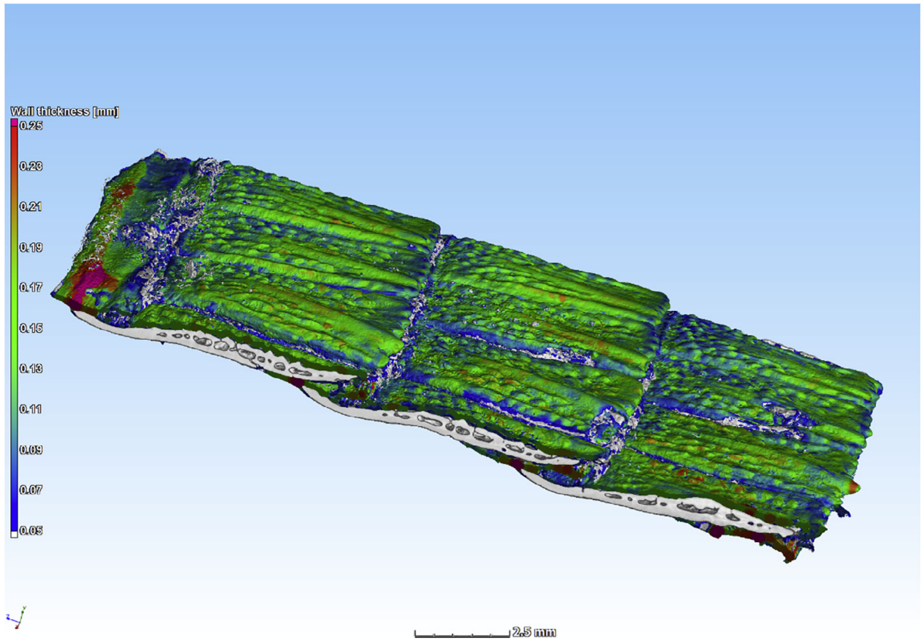

Fig. 7. Thickness map of the epidermis overlying osteoderms in the skin of an armadillo lizard, showing mean thickness of 0.15 mm. The osteoderms are seen in greyscale with their internal pores exposed in a virtual "cropped" view.

Other simulation methods are also possible either directly on voxel data or indirectly by creating suitable meshes and subsequently performing simulations in dedicated simulation packages.

4.4.1. Structural mechanics

MicroCT-based finite element analysis has become a widely used tool in bone biomechanics [97] and has been in use for a number of years. However, until recently this method required remeshing of the obtained microCT data, for extraction to simulation software tools. This step may by prone to human bias or errors of various kinds, and often requires down-sampling of the data. Recently, it became possible to eliminate this source of potential error by directly applying simulated loads to the microCT data using a simplified linear elastic finite element code which works directly on voxel data without any need for remeshing. This direct method uses a boundary-immersed finite element code and its use for biological materials was demonstrated in detail in the supplementary material of a study of snake fang mechanics [63] and one result from this work is shown in Fig. 8.

4.4.2. Permeability

Similar to the previous section, permeability simulation is possible directly on voxel data. The use of microCT images for 3D permeability simulation has previously been described in [98]. Similar

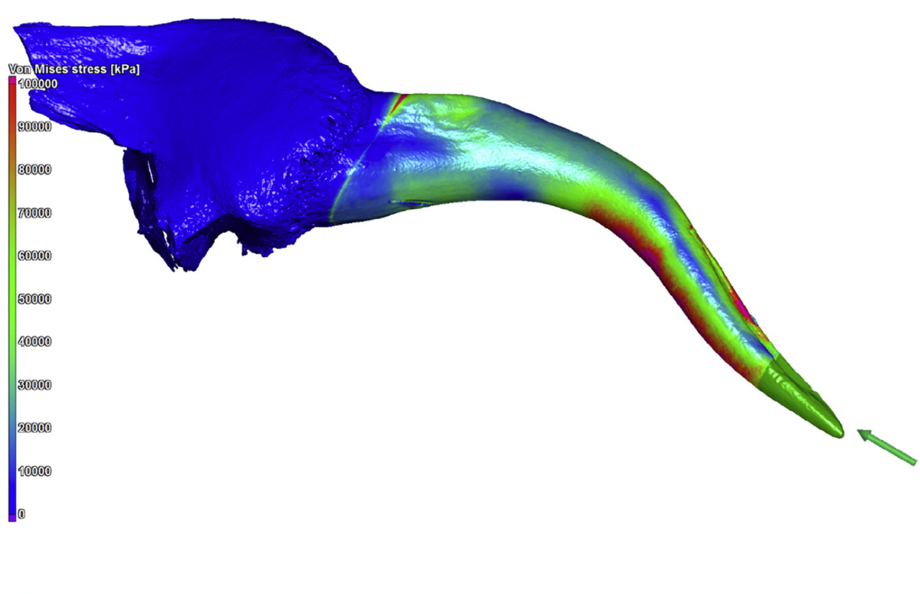

Fig. 8. Structural mechanics simulation on a snake fang, taken from [62,63]. Blue and green regions indicate fixed and load regions respectively. This simulation was compared to physical compression tests and failure occurred at the high stress areas – a live X-ray video of the compression is shown in supplementary material in [63]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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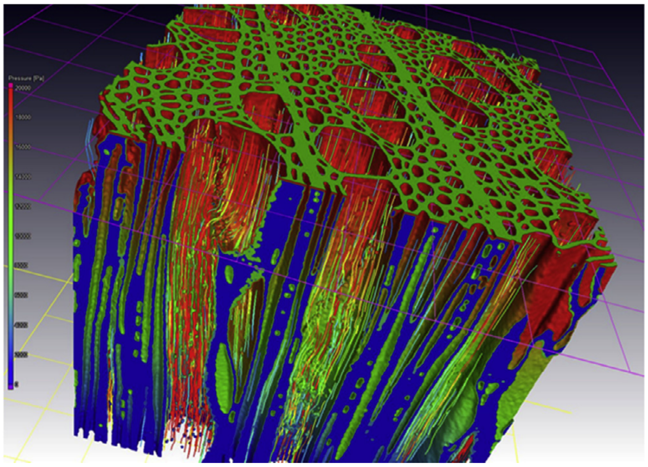

Fig. 9. Permeability simulation in wood microstructure, taken from [93]. In this simulation based on laminar flow of water, flow paths and velocities can be visualized and absolute permeability values can be obtained.

to the discussion in the previous section, this required a remeshing step in the past, but recently direct voxel-based simulation is possible. This simulation is based on laminar Stokes flow, using a two-phase system of flow in pore spaces. This is demonstrated on the microstructure of wood in Fig. 9, with color-coding by relative pressure and flow paths shown for highest velocity flow. This method was recently used in the comparison of different additively manufactured lattice structures meant for bone tissue engineering [91], to compare the differences in permeability of different lattice designs. It has not been widely used in biomimetic research yet, but as most biological materials are porous in nature, this may find particular use, for example, for the investigation of natural filtration systems.

4.5. Trends in biomaterials and biomimetic research

4.5.1. Soft-tissue imaging and staining

An increasing trend is the use of staining for visualization of soft tissue in microCT data. This might be useful for biomimetic research especially for visualization of structural architectures in softer tissues, not only dense material such as bone. The combined functions of soft and hard tissues are often critical to the function, such as the interaction between hard scales and soft underlying tissue in fish armour for protective capacity. A number of staining agents are commonly available and can easily be applied to tissues. While osmium tetraoxide ($OsO4$) has long been among the most widely used contrast-enhancing chemicals, it has now been replaced by more cost-effective, non-toxic staining agents including iodine [99,100], phosphotungstic (PTA) and phosphomolybdic (PMA) acid [101]. Despite the significant advantages that soft-tissue imaging might have, several drawbacks are associated with the technique. Firstly, staining agents, particularly PTA and PMA, might be difficult to remove from samples [99–101] and could be considered unsuitable for valuable samples (e.g., museum specimens). However, protocols to remove staining agents from tissues are available and currently receive much attention in literature [100,102]. Secondly, several staining agents possess a higher osmolarity than biological tissues, which might cause tissue shrinkage [103,104]. Shrinkage might negatively affect morphological analysis, especially those involving quantitative measurements, and its effects should be considered especially if comparisons are made across studies or samples.

4.5.2. Additive manufacturing

Additive manufacturing (3D printing) is increasingly being used to complement biomimetic studies and assist in understanding biomaterials, especially for mechanical testing of biomaterials, as reviewed recently [14]. This is usually done either through the use of 3D printed models which are then subjected to mechanical tests, often in polymer or resin materials. As mentioned in the previous section, staining allows the visualization of both soft and hard tissues in the same scan data, which can then be used to manufacture replica models with different materials using multimaterial printers.

In addition to polymers and resins, additive manufacturing of metals (especially using the technique of laser powder bed fusion) is gaining acceptance even for critical applications in aerospace and medical applications but has not yet been used in biomimetic studies until recently. Fig. 10 illustrates an example in which biomimetic lattice-shell structures were produced by laser powder bed fusion in titanium alloy. This study created models based on a biological system with variations in the bone architecture (i.e., strut thickness and shell thickness), allowing an improved understanding of the combined properties of the different lattice-shell combinations [105]. The growth of additive manufacturing technology is fast expanding the capabilities of biomaterials science and biomimetic research such as this example and clearly holds great promise.

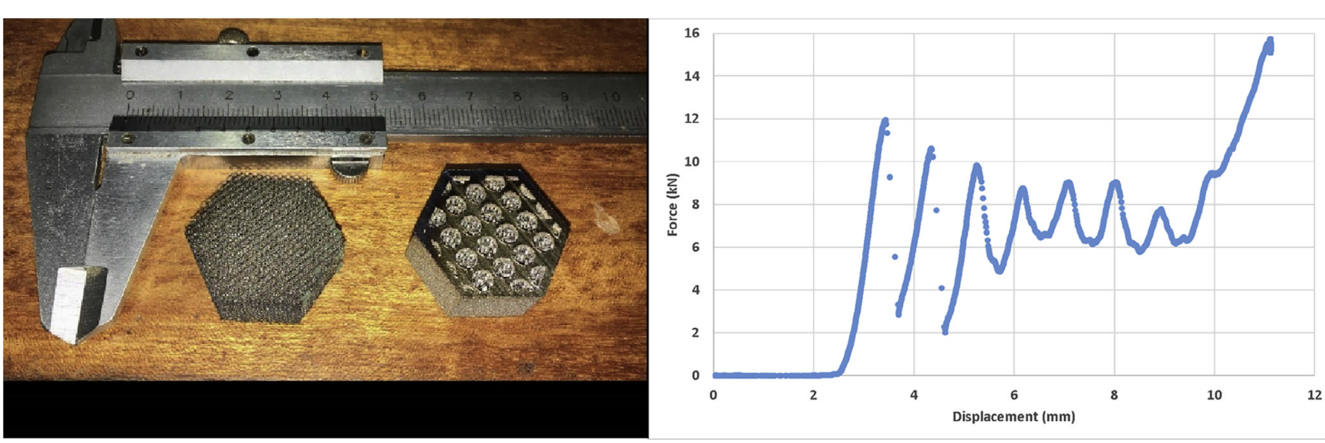

Fig. 10. Biomimetic samples produced by additive manufacturing (laser powder bed fusion) of titanium alloy, based on information obtained from microCT data of glyptodont osteoderms. Force-displacement curve shows typical yielding behavior of lattices allowing energy absorption. First yielding occurred at 12 kN for a 28 mm wide and 12 mm high sample.

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The field of additive manufacturing is growing rapidly, but one area in particular is gaining significant attention at present: the area of topology optimization. This method is based on the optimization of shapes of components according to simulations. These parts also often incorporate lattice designs (scaffolds) and result in complex-shaped functional components which can be produced by additive manufacturing. Topology optimization in this context makes use of load simulations to determine areas where highest and lowest stresses are seen due to expected maximum loads on the component and removes material in those areas where lowest stresses are found. The aim is to produce lighter parts with almost the same strength and other properties compared to the traditional part, making use of the complexity that additive manufacturing allows [106,107]. These topology optimized parts often have curves and shapes which give them an "organic" or "bionic" shape and the additive manufacturing community very often refer to these as biomimetic designs, despite the lack of biological input in the design process. We propose that these be referred to as bionic designs rather than biomimetic, unless input is derived from a natural system in one way or another. An example of a topology optimized part in which the entire topology was subsequently latticed is shown in Fig. 11. These designs hold great potential and there is a possibility to include biological input into the design process to further enhance this potential and improve these types of designs.

4.5.3. Digital repositories for microCT data

It should be mentioned that as the accessibility and use of microCT increases, the availability of open-access data sets also increases. Many researchers are depositing their data in an ever-growing number of online repositories such as Digimorph, Morphosource, Gigascience, and others. This allows researchers with limited funding to freely obtain suitable data sets and work on these for bio-inspiration irrespective the availability or cost limitations of accessing microCT scanning facilities. It might occur that high quality microCT data is used for other purposes than bio-inspiration, and that much untapped potential for biomimicry research lies in existing microCT data.

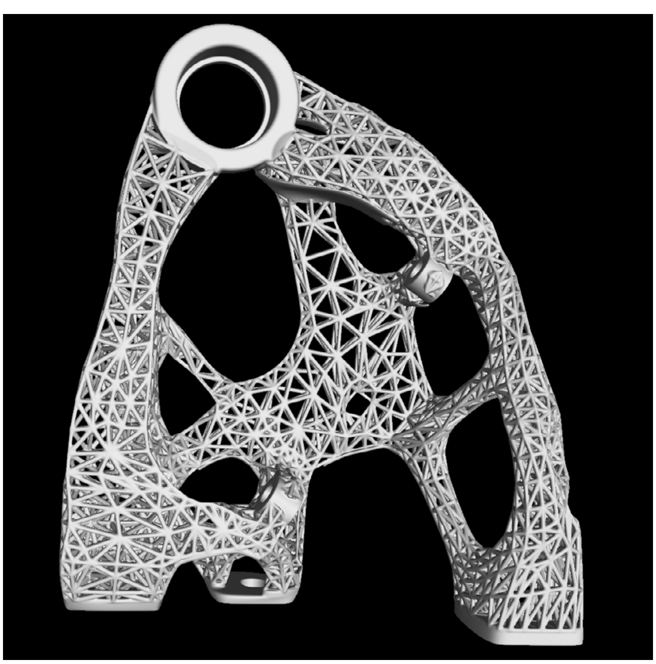

Fig. 11. Topology optimized and latticed "bionic" design for additive manufacturing, often referred to as biomimetic despite the lack of direct biological input in the design process. This part was printed in titanium alloy for use in a lightweight experimental vehicle [108].

4.5.4. Keeping up to date with latest developments

There are constant improvements in microCT hardware and software, which can be appreciated when attending international conferences dedicated to this rapidly developing field, such as the International Conference for the Tomography of Materials and Structures (ICTMS) and the Tomography for Scientific Advancement conference (TOSCA). User-group meetings of hardware and software vendors also allow a useful insight into the latest developments. Academic multi-user microCT laboratories keep up to date with latest capabilities and therefore it makes sense to use these types of facilities. Regular review articles also are helpful in demonstrating the state of the art, and keeping up to date with rapidly developing techniques. A dedicated journal for the technique is currently lacking and might emerge in the next few years.

4.5.5. New design approaches

The use of biological input in designs to solve socio-economic problems was first discussed using the Russian problem-solving approach called TRIZ [109]. This approach recently has been further developed to generate a database of biomimetic materials and use this for solving engineering problems using biomimetic designs [110]. This approach is especially important as most engineers or designers have no biology background and biologists might provide unique insights into optimized natural systems for a particular application [111]. In recent work focusing on cellular materials, the function-structure relationships of various cellular designs were classified according to their possible functional uses [112]. Future design approaches might make use of "design rules of nature", and for structural applications will definitely make use of the complexity allowed by additive manufacturing.

5. Conclusion and perspectives

This review placed the use of the emerging non-destructive imaging technology of X-ray microCT and its latest capabilities in context in the fields of biomimicry, biomaterials science and biomimetics. The technique finds particular use in studies related to structural biomimetic engineering and biomaterials science where the structure is relevant to the observed properties. It is primarily a microscale imaging technology, but macroscale (mm scale) and sub-micrometer features may be accurately visualized and measured using different types of microCT or even medical CT systems.

MicroCT is a particularly useful technique when the structure needs to be visualized non-destructively (e.g., brittle or very soft materials) or when the 3D structure is of particular interest and requires quantification. X-ray microCT has developed and evolved into more than an imaging tool thanks to developments in hardware and software tools, providing a unique 3D insight and perspective into the structure of natural materials and can assist in understanding their behavior, through advanced measurements, simulations and 3D printing technology. These advanced analyses, many of them being discussed in this review, are not yet widely applied in this community, despite the great potential they may hold. It is now possible to learn more from natural structures and to optimize the design of biomimetic devices and biomimetic engineering structures using direct and quantitative insight from 3D data of the natural structures.

Despite its many advantages, microCT has important limitations which need to be considered. As mentioned above, it is limited to mostly microscale feature sizes, and therefore does not provide a holistic solution to (bio)materials analysis across all

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length scales. For more detailed structural analysis into the nanoscale, other imaging technologies need to be combined with microCT. The second limitation is that no chemical analysis is included, which requires the user to have some knowledge of the material properties, or requires combinations of analytical techniques to provide further insight. The third limitation, is that it is a relatively new technology requiring significant expertise to obtain high quality data, especially in terms of scan parameter variations which are unique to every specimen and image processing which are unique to the type of data obtained. The result is that not all scans are high quality, not all data are useful and often data processing may be very time consuming.

There are a number of factors influencing the current increasing utility of microCT in biomimetic studies: the technique is becoming more widely used as its availability increases through university laboratories, associated computing hardware and software tools are improving and developing constantly, and alongside this, 3D printing and additive manufacturing technologies are growing significantly. These all combine to place microCT at a unique position crucial to provide the required inputs into biological materials studies and biomimetic engineering studies. This will most definitely provide huge benefits to the field of biological materials science and biomimicry, and the most exciting of these capabilities were highlighted in this review paper with examples.

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Disclosure

The authors declare no conflicting interests.

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