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Stereoscopic light-microscopy in biology – A review

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A b s t r a c t : The contribution provides a brief overview of stereoscopic photography in biology-related light-microscopy. It further raises the question, in how far this optical technique can be useful for the solution of biological problems. Production of stereo-pairs can be carried out in two different ways: (1) by photographing the object from two horizontally displaced perspectives; (2) by generating two semi-images from a single photograph with the help of specific computer software. Based on several impressive image examples it can be concluded that stereoscopic methods represent an appropriate visual support with regard to structural investigations, where spatial information has to be evaluated as highly essential.

K e y w o r d s : Stereoscopy, light-microscopy, biology, stereo-pair, semi-image, viewing technique, structural analysis.

Introduction

Stereoscopy represents an optical method, where two semi-images showing an object from two different perspectives are combined to a so-called stereo-pair. By application of specific viewing techniques (parallel view, cross view) the semi-images undergo a fusion process in the brain and the object appears with its spatial information (WHEATSTONE 1838, HELMHOLTZ 1910, STURM 2016a, 2016b, 2017a, 2017b). The intensity of this stereoscopic effect is expressed by a physical parameter termed deviation which describes the difference of the distances of two corresponding image points from the right edges of the pictures (SCHEIDEL 2009, STURM 2017a). High deviation increases the three-dimensional effect, whereas low deviation reduces the spatial information. However, the amount of this physical parameter should not exceed 3% of the horizontal image width, because otherwise the brain is not able to perform the fusion process anymore.

As outlined by numerous authors (e.g., GOLDSTEIN et al. 2003, CYPIONKA et al 2016, STURM 2016a, 2017a), stereoscopic imaging techniques have been well established in biological sciences in the meantime. Most considerable application of the method can be among other attested to the entomological field of research, where sensory organs of many species dispose of superficial microstructures. These objects can only be described sufficiently with the help of appropriate three-dimensional visualization (GOLDSTEIN et al. 2003, STURM 2017c, 2018a). In protozoology filigreed pseudopodia, flagella or cilia of unicellular organisms can be studied more effectively on stereoscopic photographs (STURM 2016b). Also the malacological discipline bears numerous interesting topics, where the use of stereoscopy may be regarded as valuable support. Many shells of recent and fossil molluscs are distinguished by specific extensions which are aligned towards all spatial directions and represent essential traits for species determination (STURM 2016a,

2016b). At last, three-dimensional visualization based on stereo-pairs can represent a valuable support in microbiology, botany, marine biology, histology and all kinds of medical disciplines, where the combination of light-microscopy and photography is subject to a frequent use (GOLDSTEIN et al. 2003, STURM 2018a, 2018b).

In this contribution the role of stereoscopy in biological light-microscopy is submitted to a detailed documentation. With the help of reliable photographic material major advantages and drawbacks of stereoscopic visualization are discussed.

Materials and Methods

Classical methods of stereo-pair production

According to the "classical" method stereo-pairs are produced by simply photographing the microscopic object from two different perspectives. Basically, three types of multiperspective imaging can be distinguished in light microscopy (STURM 2016a, 2017b, 2018b): The easiest way of production of the two semi-images is provided by photographing the transparent or opaque object at two horizontally displaced positions. Thereby, the amount of displacement depends on the size of the object and the microscopic magnification. In general, any horizontal shift (x) should be on the order of the object width (W): $x \sim W$ (Fig. 1a). According to the second method the item is photographed at its standard position and subsequently rotated by an angle α of 5 to 10° (Fig. 1b). After adjustment of this rotated position, the second image is produced. This technique, however, requires an appropriate microscopic equipment allowing the exact rotation of the sample holder. If such a device is not available, the microscopist may overcome this deficit by slightly lifting one edge of the sample holder (STURM 2016a, 2016b). The third technique is restricted to transparent objects and transmitted light-microscopy. Here, different focus planes are produced by varying the distance between microscopic objective and investigated structure. At each focus adjustment the object is photographed, so that an "image stack" is produced. Two of these photographs are finally prepared to the stereo-pair (Fig. 1c). The distance of two focus planes (z) depends on the size of the object but should be on the order of 2 to 10 µm.

In order to generate optimal stereo-pairs both semi-images should show identical illumination and depth of focus. Application of "classical" stereoscopic imaging techniques is limited to immotile objects such as all types of shells, spores or pollen. In the case of living objects being marked by a certain degree of motility (e.g., unicellular organisms), computer-aided generation of stereo-pairs outlined in the next section has to be carried out.

Computer-aided generation of stereo-pairs

With the help of modern computer software stereo-pairs can be simply produced from a single photograph of the investigated object. Computer programs such as PICOLAY developed by H. CYPIONKA (RAAP & CYPIONKA 2011, CYPIONKA et al. 2016) are based on the theory that, in the case of vertical incidence of light, objects become continuously darker from their front to their back. This gradient of brightness, however, is used for the computation of a so-called object-depth map (ODM), on the basis of which certain spatial information of the object can be modeled. Partly visible surfaces and edges can be extrapolated to pre-defined image depths by application of specific procedures of rendering. At

the end of this process the photographed object becomes rotatory by a certain amount (1 to 10°) and can be visualized from two different perspectives, resulting in the stereoscopic image pair (Fig. 2). Besides the considerable advantage of recording living organisms, the computer-aided method can be also applied to old photographic material, where spatial information can probably help to answer unsolved questions.

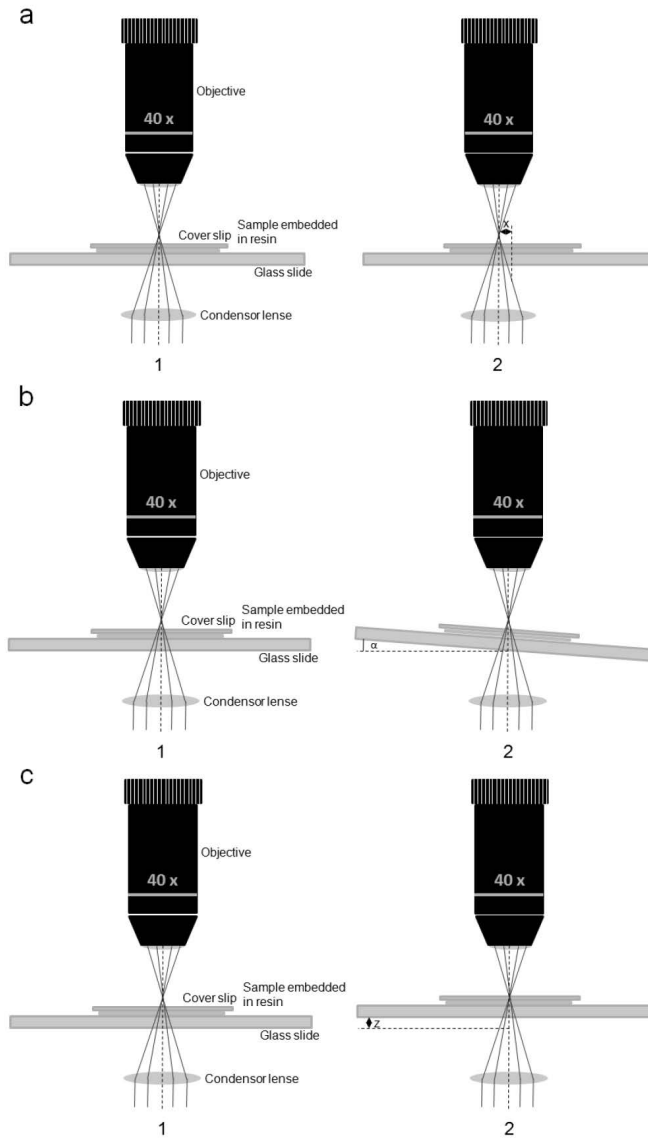

Fig. 1: "Classical" methods of stereo-pair production which are described in detail in the scientific literature: (**a**) horizontal displacement of the object, (**b**) rotation of the object, (**c**) variation of the focus plane (restricted to transparent objects).

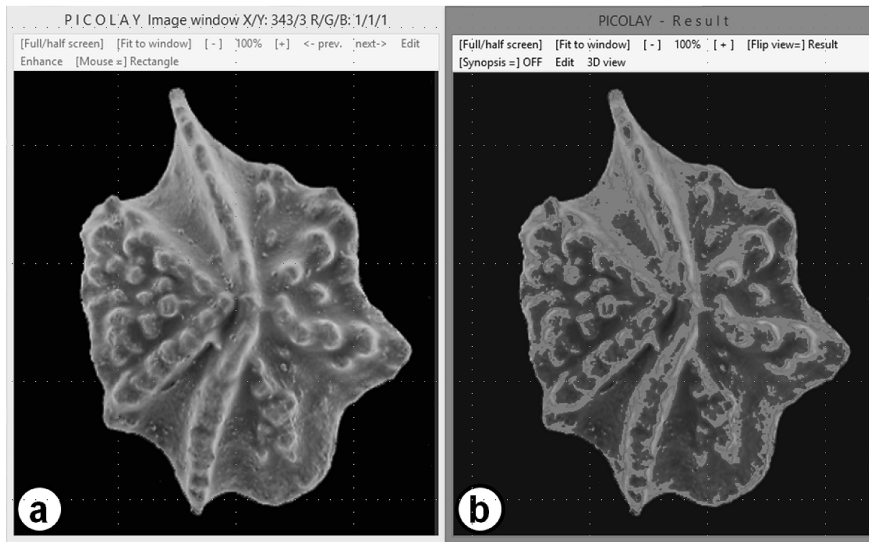

Fig. 2: Main windows of the computer program PICOLAY which was developed for the generation of stereoscopic images from single photographs: (a) input window, (b) output window with generation of the object-depth map.

Results

Several examples illustrating the essential role of stereoscopic micro-photography in biological sciences are summarized in Figs. 3 to 8. As already raised in the introduction, protozoology represents a preferential application field for stereo-imaging, because respective organisms are usually observed under the light-microscope and dispose of three-dimensional structures which require special visualization techniques. This circumstance is impressively demonstrated for the trumpet animal *Stentor* sp., representing a sessile freshwater ciliate with a size ranging from 150 to 500 µm (Fig. 4). This organism is characterized by its circular mouth surrounded by numerous rows of cilia which serve for the supply of the cell with nutritive substances. With the help of stereo-photography proportions and shape of the mouth as well as movement of the cilia can be studied in a more effective way. Another strategy of food intake is pursued by amoeboid organisms like that depicted in Fig. 6. These unicellular objects use numerous pseudopodia to enclose their bait. After inclusion of the obstacle into the cell, phagocytotic processes consisting of a dissolution of the bait are started. Besides unicellular animals characterized by heterotrophy also unicellular plants following an autotrophic way of life represent desirable motifs for stereoscopic micro-photography. Highly impressive organisms belonging to this group are the diatoms with their bipartite shells consisting of the lower hypotheca and the upper epitheca. Shape and superficial structure of a given shell are main criteria of species determination and can be well presented by stereoscopic visualization techniques (Fig. 4).

In the case of multicellular organisms of microscopic size stereo-imaging can also provide a valuable optical support. This circumstance among other proves true for the freshwater

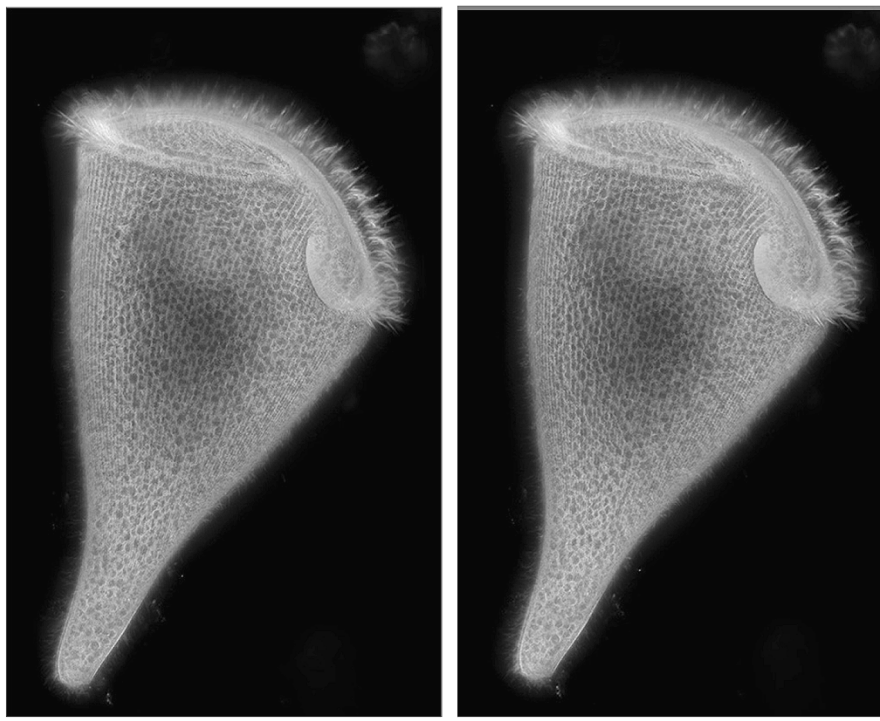

Fig. 3: Stereoscopic photographs of the trumpet animal *Stentor* sp, representing a sessile freshwater organism with partly massive occurrence (height: 200 µm, single images: Wim van Egmond).

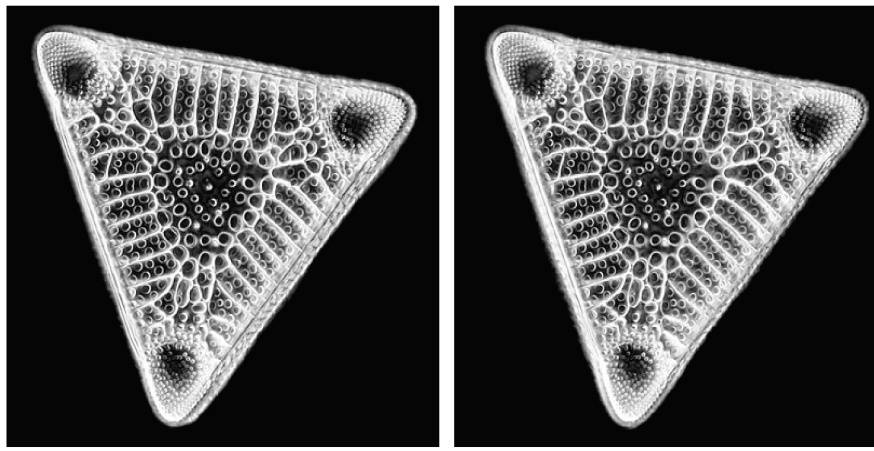

Fig. 4: Stereoscopic images of a triangular diatom which represents a widely distributed unicellular organism of small freshwater habitats with sumptuous stock of submerged vegetation (width: 250 µm, single images: Wim van Egmond).

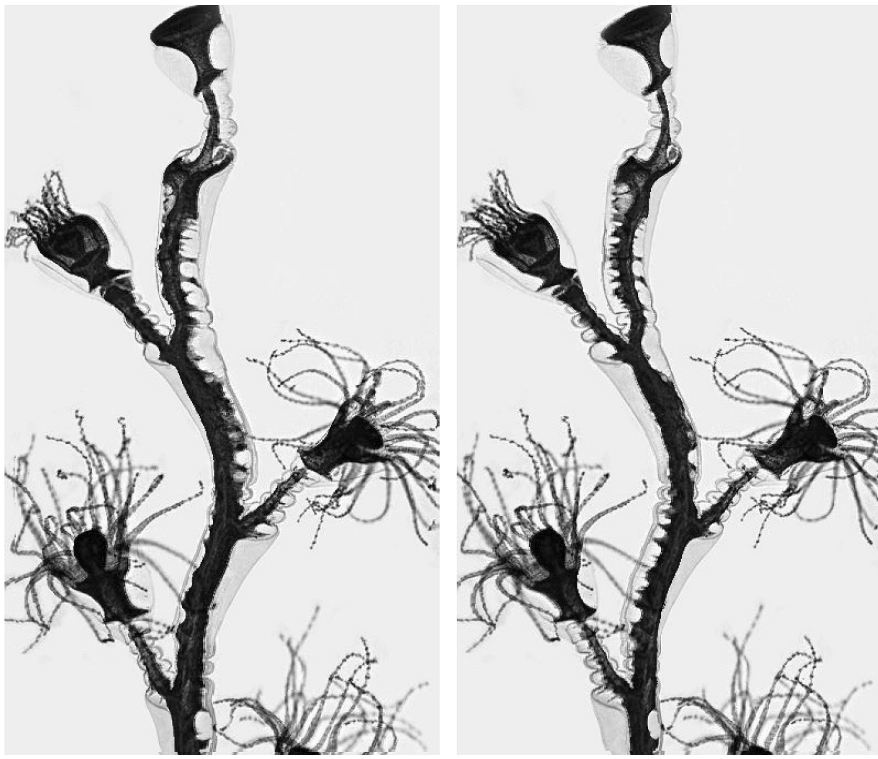

Fig. 5: Stereo-pair showing the freshwater polyp *Hydra* sp. under the light-microscope (height: 0.5 mm).

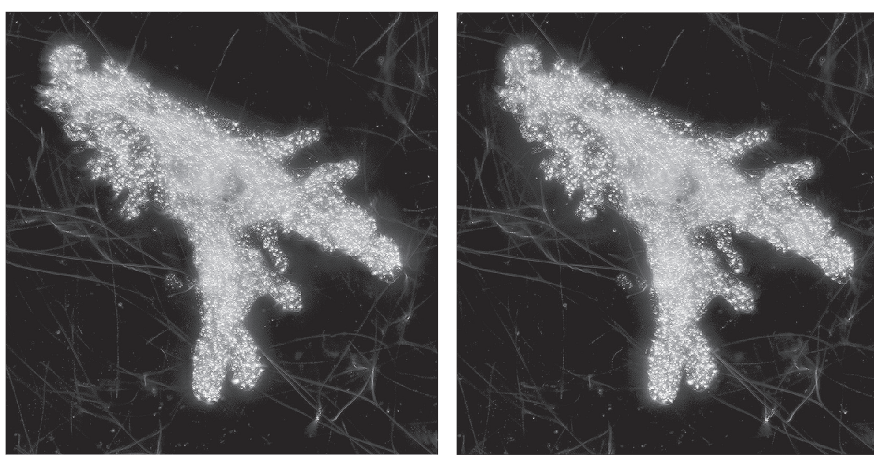

Fig. 6: Stereoscopic images of an amoeba with its irregular pseudopodia extending into all spatial directions (width: 150 µm, single images: Wim van Egmond).

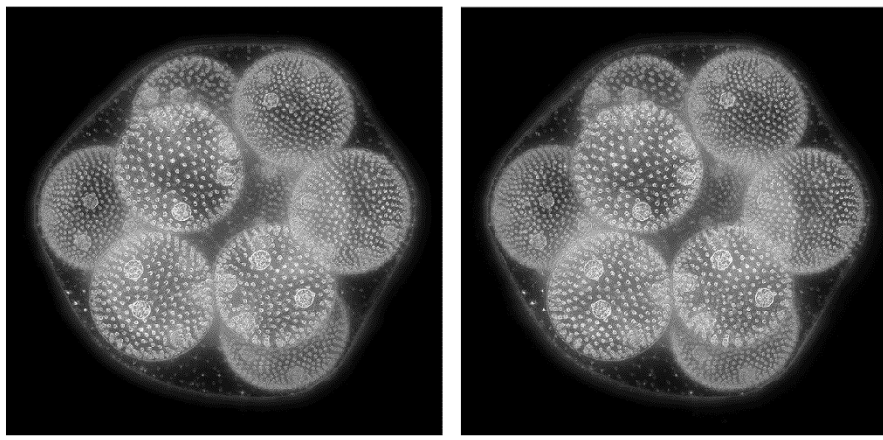

Fig. 7: Stereoscopic view on the multicellular green alga *Volvox* sp. (diameter: 250 µm, single images: Wim van Egmond).

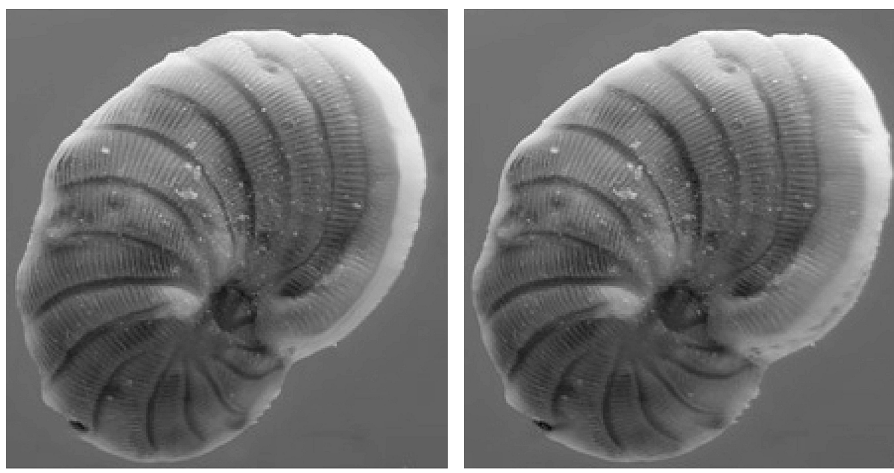

Fig. 8: Spiraliform tests of a recent foraminifer (diameter: 300 µm).

polyp *Hydra* sp. depicted in Fig. 5. The cnidarian animal usually forms colonies including numerous individuals which carry out the food intake from all possible directions. In the stereoscopic image spatial orientation of single individuals and their tentacles can be visualized appropriately. Another multicellular organism is represented by the green alga *Volvox* sp. forming globular objects. The spatial extension of these colonies can be well estimated in the stereo-photograph (Fig. 7). The last example provided in this brief overview is the shell of a recent unicellular foraminifer which somewhat reminds on the shell of a gastropod or cephalopod, but differs from them by at least one order of magnitude (Fig. 8). Here, stereo-imaging can provide some additional information on the depth and superficial characteristic of the investigated structure.

Discussion

In the past decades various optical methods have been established in biosciences as valuable support for the solution of numerous research questions (STURM 2015, 2016a). Development of innovative microscopic techniques and devices has resulted in a continuous increase of the resolving power and quality of visualization, allowing a more detailed documentation of diverse microstructures. Although stereo-photography represents a rather traditional optical procedure, which was already invented in the 1850s (WHEATSTONE 1838, SCHEIDEL 2009, STURM 2016a), its breakthrough in natural sciences succeeded not until 100 years later, when three-dimensional imaging gained more and more importance (STURM 2009, 2011, 2015, 2016b, 2017a, 2017b, 2017c, 2017d).

In biological disciplines such as zoology, botany, histology or microbiology light-microscopy is used for both educational and scientific purposes. Currently, acquisition of a high-quality light-microscope is also possible for hobby microscopists and smaller working groups, because production costs have been strongly decreased in the past decades. This prize development can be also attested for the photographic equipment being necessary for the generation of professional stereo-images (SCHEIDEL 2009, STURM 2016a, 2016b). As reported in earlier studies, photographs of sufficient quality can be alternatively obtained with the help of a cell-phone camera, whose lens is centrally placed over the ocular of the microscope (STURM 2016b). Hence, stereo-photography finally presents as neither expensive nor time-consuming method which may be regarded as considerable advantage with respect the three-dimensional visualization procedures (e.g., holography; STURM 2017c, 2017d).

Other advantages occurring in association with stereoscopy include: (1) the easy learning of autostereoscopic viewing techniques, so that optical devices such as stereoscopes or stereo glasses become unnecessary after a certain while; (2) the easy storing and sending of digital stereo-photographs to research colleagues; (3) the subsequent preparation and improvement of old two-dimensional photographs. Besides these positive aspects, however, microscopic stereo-photography also includes some drawbacks, among which the limited three-dimensionality is certainly most striking. This means that the whole object with its front and back can not be visualized by a single stereo-pair, but requires lots of stereo-pairs that have been produced from perspectives around the object. Classical stereoscopy can be influenced by both horizontal and vertical parallaxes which disturb the fusion process of the semi-images to a certain degree. Therefore strict rules have to be kept during picture recording (SCHEIDEL 2009, STURM 2016a, 2016b).

Summing up, it may be concluded that stereoscopic photography has found its place in biology meanwhile and will play an essential role in microscopy in the future. For this, a ambitious communication of the technique and its advantages has to be pushed ahead.

Zusammenfassung

Stereoskopische Lichtmikroskopie in der Biologie – Eine Überblicksdarstellung. Der Beitrag liefert einen kurzen Überblick zur stereoskopischen Fotografie in der Lichtmikroskopie mit biologischem Bezug. Er wirft in weiterer Folge die Frage auf, inwiefern diese optische Technik von Nutzen für die Lösung biologischer Problemstellungen ist. Die Herstellung von Stereopaaren kann auf zwei verschiedene Weisen erfolgen: (1) durch Fotografie des Objektes aus zwei horizontal verschobenen Perspektiven; (2) durch Generierung zweier Halbbilder auf Basis einer Einzelfotografie mithilfe

spezifischer Computersoftware. Basierend auf etlichen eindrucksvollen Bildbeispielen kann geschlussfolgert werden, dass stereoskopische Methoden eine geeignete visuelle Unterstützung in Hinblick auf strukturelle Untersuchungen repräsentieren, wo räumliche Information als in hohem Maße essenziell zu betrachten ist.

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