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A new versatile compression chamber for examination of living microorganisms

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ABSTRACT: A new type of compression chamber for microscopic studies of living microorganisms is described. This broadly applicable instrument allows controlled slowing down, turning and compression of vagile organisms. It has been developed for use with different types of microscopes, especially the modern inverted versions. Due to the extremely flat design, illumination according to the Köhler principles is guaranteed. Therefore, all modern procedures such as bright-, darkfield, phase-contrast, differential interference contrast, polarized and fluorescent microscopy can be carried out. Once focused, parfocal objective lenses (even oil immersion high power objectives) can be interchanged without refocusing. The chamber is composed of three basic elements: (1) baseplate, with cylindrical head-piece, (2) compression ring for vertical adjustment, (3) rotor, non-threaded and gliding on viscous silicon. An upper and a lower circular coverslip is permanently glued to the rotor and baseplate respectively. Preparation and interchange of the specimens are rapidly done by lifting and subsequent insertion of the non-threaded rotor. Thus, the new chamber is an almost non-evaporating closed system and the organisms to be studied are preserved alive for many hours. Except when high power magnifications are used, the principles, on which the compression chamber is based, allow various modifications in outfitting and design, such as transformation into a continuous flow system for fresh- or seawater, or exchange of media at choice. The chamber and its function is compared with similar instruments, such as the rotocompressor and the Spoon microcompressor.

INTRODUCTION

The study of living microorganisms, microalgae, protozoa, nanoplanktonic and meiobenthic or parasitic specimens depends essentially on suitable preparations. Fast moving organisms or cells have to be slowed down, but sedative agents should be a last alternative. Quick preparations on covered glassplates are usually "oneway streets"; retrieval of organisms is not assured, purposeful manipulations are not possible, and the observation period is very limited. Therefore, microchambers are indispensable tools, especially for long-term and cinematographical studies. A variety of simple or more or less complicated designs have been described elsewhere (Heunert & Uhlig, 1966; Heunert 1970; Spoon, 1978) and are on sale, very often at immoderate charges.

The various types are designed, almost without exception, for upright (transmitted or reflected) light microscopes. However, in biological research and routine work the inverted type of microscope is increasingly used, utilizing the tendency towards sedimentation of organisms and consequently, the considerable advantages for the detailed examination on a mainly two-dimensional level. The principle of inverted

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microscopy was originally introduced by Utermöhl (1931), particularly for studies on marine plankton. The advantages are (a) high stability with stationary tubes and stages, (b) retention of image sharpness even at high power magnifications and (c) large working distances, allowing the use of high chambers, vessels or dishes.

Especially for microscopical live examinations, the mobility of the more or less vagile microorganisms can be slowed down or cut short by a suitable compression chamber like the "roto-compressor". According to Spoon (1978), this never patented, precise instrument was developed about 40 years ago by A. A. Schaeffer (Biological Institute of Philadelphia, USA). It is preferred by many protozoologists, meiobenthologists and planktologists in the original or in modified versions. Unfortunately, the specific design of the roto-compressor does not allow an adaption to inverted microscopes.

In order to compensate for this problem and to combine new features and advantages as much as possible, a new kind of microchamber was developed.

DESCRIPTION AND FUNCTION

The principle of the construction is illustrated by a cross-sectional view in Figure 1. The chamber is composed of only three basic elements (Fig. 2): (1) The baseplate (M) with a cylindrical head-piece and an external thread and the coverslip aperture with an

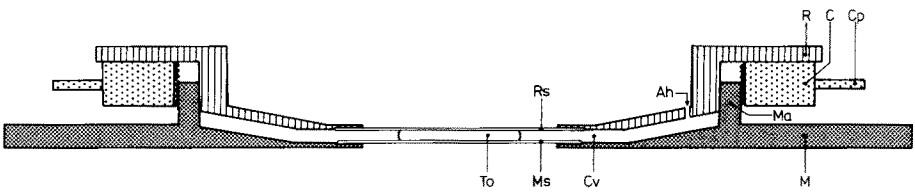

Fig. 1. Cross-section of the new compression chamber for studying living microorganisms. Ah: airhole. C. compression-ring. Cp: pin for turning compression-ring. Cv: chamber volume. M: baseplate. Ma: head-piece of the baseplate. Ms: lower coverslip. Rs: upper coverslip. R: rotor. To: compressed medium with organisms. For further explanations see text.

extremely flattened support surface, (2) the compression ring (C), with a wide upper guide surface and an internal thread, fitting into the head-piece of the baseplate, (3) the rotor (R) with coverslip aperture, horizontally gliding upon the lubricated upper guide surface of the compression ring; vertically, within the internal cylinder wall of the head-piece. Both guide surfaces are lubricated with viscous silicon.

The two circular coverslips (Ms and Rs) of 0.17 mm thickness each, are inserted and permanently glued to the baseplate and rotor, respectively, using a quick-dry commercial embedding medium. The vertical adjustment of the rotor is given by turning the compression ring to the right or to the left. At the lower edge of the rotor, two small boreholes (airhole Ah) admit air exchange between the chamber cavity (Cv) and the atmosphere when the rotor is inserted or lifted off.

The chamber is quickly opened by withdrawal of the unthreaded rotor. Single or groups of organisms are placed with a small drop of water onto the center of the coverslip (Ms). Depending on the thickness of the organisms to be studied, the compression ring is

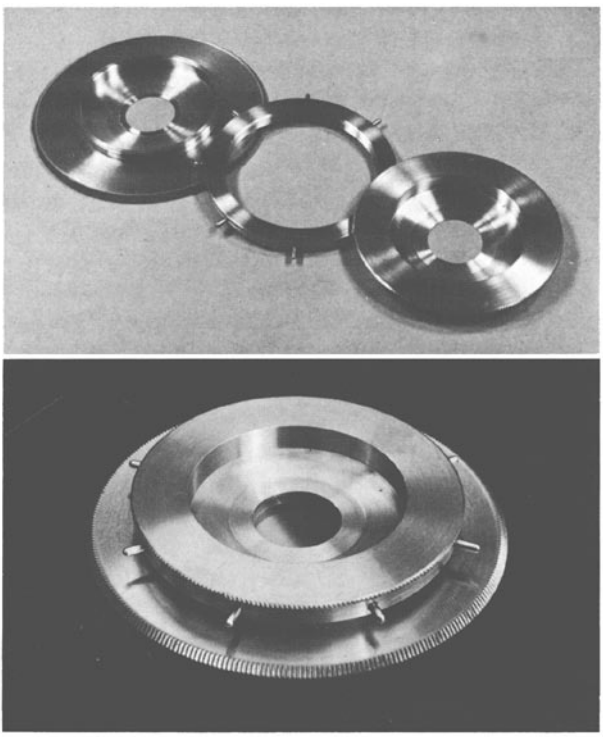

Fig. 2. Views of the opened (above) and closed (below) compression chamber. Above: baseplate (left), compression ring (middle) and rotor (right)

threaded at a higher or lower level. Subsequently, the rotor is inserted into the headpiece where the cylinder glides down, according to the chosen degree of compression. Here the rotor coverslip (Rs) contacts the drop of water. With clockwise turning of the compression ring by means of the pins (Cp) and simultaneous adjustment (fixation) of the edged rotor the organisms are increasingly sandwiched and flattened between the coverslips; if desired, without horizontal deflection. Besides, rotund or spheroidal organisms or cells can be rolled from one to another side by cautious use of the rotor.

Due to the closed system there is almost no evaporation of water within the chamber, an especially important factor when studying marine organisms. Therefore softly squeezed organisms can be well preserved for hours. Furthermore, the chamber is transformable into a flow system for fresh- or seawater, or for any other desirable medium, if the two airholes of the rotor are used as capillary inlet and outlet respectively.

Because of the extreme central flatness of the baseplate (support surface of the lower coverslip less than 0,5 mm thick) the lower coverslip (Ms) is positioned just above the level of the microscope stage. Therefore, in application to inverted microscopes, this low profile design allows switching of any parfocal objective into position without refocusing even changing from low to high power magnifications with a sextuple revolving nosepiece; vice versa, the specific objective to be used does not have to be raised and lowered. On the opposite side, the condenser fits into the spacious recess (deepening) of

the rotor. Therefore, universal application of even oil immersed condensor lenses is possible.

The flat design of the chamber guarantees optimal illumination according to the Köhler principles. Thus, all studies can be done at bright – or darkfield, phase-contrast, differential interference contrast (Nomarski) as well as in polarized or fluorescent light. Additionally, the low profile permits survey of the total coverslip area from edge to edge.

The chamber is applicable also to conventional upright microscopes, although it has one slight restriction: with turreting high power objectives, the lenses may touch the inner edge of the rotor. Therefore, raising of the objectives or respectively lowering of the microscope stage and refocusing will be necessary.

The extremely flat chamber of relatively high precision provides the qualification for exact studies of internal structures and specific measurements of the size of small microorganisms ($<100\,\mu m$) at high power magnifications ($>\times 45$). In this case, the central coverslip apertures of both the rotor and baseplate are size-limited to avoid the action of capillary forces and, consequently, the abrupt and complete flattening of the organisms. For reasons of stabilization and precise manufacturing, the baseplate (M) is composed of brass. It is also ventilated to use transparent, inert plastic material in combination with brass. In this case, the chamber would consist partly or completely of corrosion-resistant material. A plastic chamber could easily be designed, if there were not such high demands for precision. Investigations on larger specimens, for instance, could be studied sufficiently at lower magnifications.

At present, the compression chamber can be used successfully for slowing and compressing microorganisms up to a thickness of about $10\,\mu m$. For example, exact determinations of cell thickness are accomplished by focusing the upper and lower coverslip area and observing the distance on the dial indicator of the focusing wheel of the microscope. Combined with planimetry, more reliable data on cell volumes and biomass calculations are possible.

On completion of an examination, preferably under stereo-microscopical control, the micro-chamber can be opened very quickly by lifting off the rotor. Immediately, a new sample can be prepared or the specimens to be examined can be exchanged. After insertion of the rotor the sample is ready at once for observation, provided that the compression ring is not turned, i.e., the previously set degree of compression has not been changed.

DISCUSSION

The principle of the new compression chamber leaves full scope for the development of a variety of modifications. When used with conventional upright microscopes, the general profile design needs only to be enlarged (internal diameter of the chamber cylinder from 50 to about 70 mm) so as to avoid contact with turreting objective lenses. In that case the lower coverslip could be replaced by a thicker glassplate. If demands for high precision (studies with oil immersed objective and condenser lenses) are reduced, the compression chamber could be partly or totally made out of transparent plastic material, which is optimal for use with seawater.

The above mentioned "roto-compressor" invented by Schaeffer has proved very successfull in the past. It is composed of 9 items and its perfect function depends on

reliable precision-tool manufacturing (6 internal or external threads). Consequently, it has a relatively high price. Its applicability is limited to conventional upright microscopy, owing to the thick basal glassplate. The specific construction does not allow this glassplate to be replaced by a coverslip of 0.17 mm thickness, which is indispensible for optical reasons.

Recently, a new "rotary microcompressor" has been described by Spoon (1978), specially designed to utilize standard slides and coverslips (22 x 22 mm or 22 × 40 mm). The Spoon microcompressor certainly has some advantages over the roto-compressor, especially the low profile design and the quick set up. There are, however, some notable shortcomings: (1) The compressor is a more or less unclosed system and higher evaporation is unfavourable for marine organisms. (2) Organisms sandwiched between the glassplates cannot be turned passively from one side to another (for instance, from dorsal to ventral) which would be an advantage in more or less spheroidal specimens. (3) Complete flatness of coverslip and glassplate, a functionally important condition, is not assured. (4) Just as with the roto-compressor, the Spoon microcompressor cannot be used with inverted microscopes. Last but not least, the design and even more the accessories of the Spoon chamber are to a considerable degree more complicated than that of the roto-compressor. Provided with only one internal and external thread it is, nevertheless, composed of at least 10 items + 4 clips + 8 screws, made of aluminium, aluminium alloy and stainless steel. Apart from the above-mentioned disadvantages, the relatively complicated arrangement results in an appropriately high cost of production.

CONCLUSION

In comparison to the above-mentioned tools the pecularities and main characteristics of the new versatile compression chamber can be summarized as follows: (1) Suitable for use with a wide range of different types of microscopes, especially inverted and conventional upright ones. (2) Optimal illumination according to the Köhler principles is guarranteed. Therefore, universal application of all modern optical procedures in microscopy, such as bright or dark-field, phase-contrast, differential interference contrast, polarized or fluorescence microscopy from lowest to highest power magnifications. (3) Quick preparation and exchange of specimens to be studied by simple lifting and subsequent insertion of the unthreaded rotor, gliding on a viscous silicon lubricant. (4) The chamber is only composed of five elements; consequently, it can be produced at a relatively low price.* (5) Due to the low profile, the total aperture of the rotor and baseplate, respectively, is open to microscopical observation. (6) When used with inverted microscopes, after a single focusing at low power, the other parfocal objective lenses can be swung into position without refocusing. Thus living organisms or cells can be studied in great detail using high power magnifications, even oil-immersed objective and condenser lenses. On the other hand, when used with conventional upright microscopes, refocusing of the high power objectives is necessary. (7) The state of compression can be changed under microscopic control, provided that the chamber is well fixed on the microscope stage. (8) The compression chamber presents a closed system allowing

* The compression chamber can be ordered from HYDRO-BIOS-Apparatebau GmbH, Am Jägersberg 7, D-2300 Kiel-Holtenau/FRG

almost no evaporation. Accordingly, it is suitable for studying marine organisms for hours. (9) Except for high power magnification, the functional principle of the compression chamber allows various modifications in its outfitting or design (fabrication with transparent plastic material, exchange of media by a continuous flow system).

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