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Fast Sample Switching Mechanism for Atmospheric Scanning Electron Microscopy and Electron Beam Irradiation Systems of Living Cells

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Atmospheric scanning electron microscopy is a technique that enables high-resolution imaging of living specimens and targeted irradiation of specific regions of interest in the sample. To produce a highly focused electron beam, a high vacuum is required inside the electron gun column. However, living specimens need to be maintained at atmospheric pressure to survive. To enable interaction between the sample and the electron beam, the sample is placed on a thin silicon nitride window, which forms the barrier between the vacuum and the atmosphere with minimal scattering of the electron beam. This allows for the imaging of the specimen through the window surface. We have developed a mechanism that allows for fast switching between the samples while the system is under operational pressure. Timing is crucial in the case of living specimens because wet samples undergoing a lengthy preparation process may dry prematurely. Typically, changing between samples can take around one hour or longer because each time, the electron gun column has to be re-pressurized and evacuated again. Our system allows for changing between samples within seconds with excellent pressure stability. We have successfully introduced and tested a hand-operated, low-cost, and scalable solution by utilizing a rotary turret system with a customized bearing arrangement and an innovative approach to standard O-ring seals.

topics: silicon nitride film, electron beam irradiation, atmospheric scanning electron microscopy (ASEM) sample switching, excitation-assisted (EXA) microscopy

1. Introduction

In recent years, the biomedical imaging field has put a high emphasis on super-resolution microscopy [1] and the possibilities of processing biological samples with external stimuli such as electron beam excitation [2]. The goal of such research is to understand the processes that occur in irradiated cells. One example of a system that is able to perform both high-resolution imaging and electron beam (EB) irradiation is the direct electron beam excitation-assisted optical microscope D-EXA [3]. It belongs to an ASEM (atmospheric scanning electron microscope) group of devices, with which a sample (living specimen) can be observed in liquid or atmosphere by using an inverted scanning electron microscope [4]. The sample is typically placed on a thin film of silicon nitride (SiN), which allows the electron beam to pass through with little scattering. The EB gun and scanning and focusing lenses are placed in a vacuum chamber underneath the sample, and an aligned optical microscope is located above it, as shown in Fig. 1. The system can be used as an inverted SEM (scanning electron microscope), a targeted EB irradiation source, and super-resolution fluorescence microscope when SiN film [5] is coated with ZnO [6]. Despite the many advantages of ASEM systems such as D-EXA, there is a serious drawback with regard to productivity in terms of specimen handling. Typically, only a single specimen dish can be observed/irradiated at a given time. The need to evacuate the EB vacuum chamber to less than 3×10^{-4} Pa, which takes 1–2 h, can be a disadvantage when many samples are lined up for processing. In the case when the drying time of the sample is very important, a several-hour wait in a cue may cause sample destruction. The same problem happens in non-focused EB irradiation systems [7], where the EB irradiation can be performed on a larger area. Figure 2 shows the principle of such a system.

A sample switching mechanism is a necessity in order to provide a higher throughput of the described systems. The motivation to develop and implement such a device is necessitated by the fragile nature of living specimens and the time constraints for processing. Our research has led to the

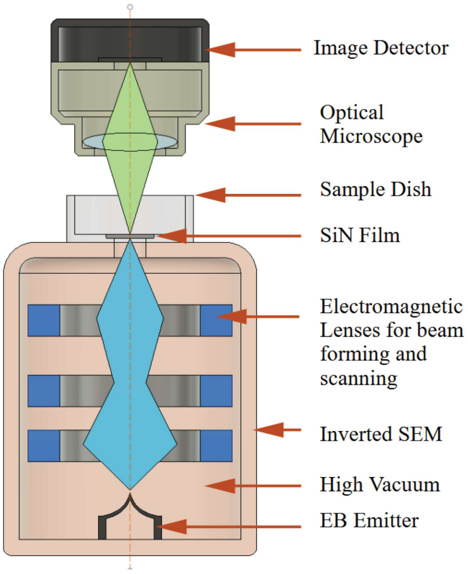

Fig. 1. Principle of D-EXA ASEM microscope.

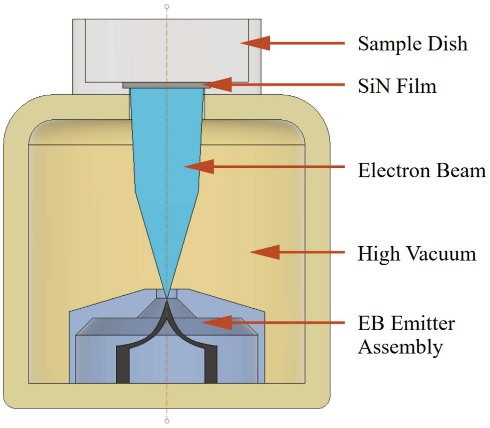

Fig. 2. Principle of non-focused electron beam irradiation system.

successful implementation of a turret-based system that can accommodate at least 2 samples. As a test for the performance (especially the seal and bearing arrangement), we have used an experimental non-focused EB irradiation system.

2. Materials and methods

2.1. Experimental EB irradiation system

EB irradiation system (APCO-AGUN30K-S) used in the development of the sample switching mechanism accepts standard 35 mm culture dishes as D-EXA microscope. The dish assembly is composed of a plastic vessel, a stainless backing disc, and SiN film [8]. The parts are bonded together using low out-gassing epoxy, as shown in Fig. 3. The prepared dishes are subsequently tested for vacuum leaks [9]. The irradiation EB current can be measured by means of a Faraday cage [10]. The choice of the measuring techniques depends on voltage level and precision [11, 12].

Fig. 3. Standard 35 mm D-EXA ASEM dish.

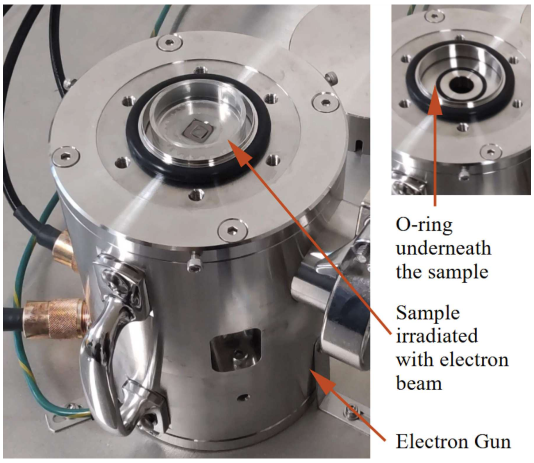

Fig. 4. Test system for EB irradiation of samples on a standard 35 mm D-EXA culture dish.

The quality of the surface of the stainless disc (in order of the roughness average $Ra = 1.6\ \mu m$ or less) is crucial as it is responsible for forming a good vacuum seal with the O-ring underneath (NBR S22.4). The O-ring is lubricated by a low-outgassing, vacuum-compatible grease and is placed concentrically in a recess on top of the EB gun cover, as shown in Fig. 4.

The necessity of using vacuum-compatible grease on the O-rings is related to difficulties in preparing a sample dish with a perfectly smooth and flat bottom surface of the disc. The epoxy, while drying, coupled with the intrinsic imperfection of the molded plastic dish, can introduce a slight warp of the disc that is 0.2 mm in thickness. During sample preparation, microscopic scratches may also appear on the surface, hence the grease plays a very important role in the vacuum seal. There is no external force holding the dish down. If it is prepared correctly, the atmospheric pressure over the surface of the O-ring

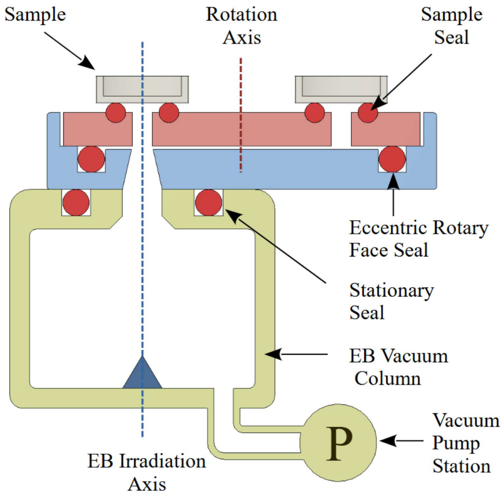

Fig. 5. Simplified vacuum system and seal arrangement.

creates an estimated $F = 40$ N force, which is sufficient to maintain a vacuum of less than 10^{-4} Pa. Same principles will apply to a switching system that can accommodate multiple samples.

2.2. Development of the sample switching mechanism

We propose a sample switching system, where multiple samples are loaded onto a turret-like mechanism with an indexing latch mechanism. The sample is selected by rotating the turret at a selected angle (dependent on the amount of samples), and the position is maintained by a latching mechanism that prevents accidental change or drift over time.

2.2.1. Vacuum system and outline of the necessary seal placement

The biggest challenge and crucial key point of the proposed system is the vacuum seal arrangement. For feasibility and low-cost maintenance, standard NBR-type O-rings are used [13]. Figure 5 shows the simplified diagram of the seal assembly. The stationary seal O-ring (G-80) and sample O-rings (S-22.4) are used in a stationary manner (no relative sliding motion occurs), however, the eccentric O-ring (G-60) requires vacuum-compatible grease lubrication for smooth operation with minimized friction.

Easy access for maintenance and cleaning is an important aspect of this system. It is possible that a SiN film can break, and its parts or the sample itself can contaminate the internal surfaces of the mechanism. For that reason, easy access is provided by using exclusively atmospheric pressure and sealing surface quality to maintain a high vacuum.

2.2.2. Design of the sample turret and bearing assembly

The proof of concept of the switching system consists of two sample positions on the turret. This choice has been made to start with the smallest feasible mechanism that can be scaled up further as the research progresses. In order for the turret to be conveniently hand-operated, a simple thrust bearing arrangement has been incorporated into the design. As shown in Fig. 6, the 5 mm steel balls are used as a thrust bearing, and by rolling in races concentric to the turret's rotation, they also provide precise centering of the rotation. No radial load other than hand-induced force acts on the bearing; the depth of the races has been set to 0.5 mm, providing radial capacity that suppresses the possibility of the bearing shifting under radial load.

The eccentric O-ring (G-60), under atmospheric pressure acting on the turret, is carrying an axial force of ~ 280 N. If the bearing was not present, it would cause the axial deformation measured to be 0.38 mm (by that amount, the surface of the turret would be lowered under atmospheric pressure if the EB column were evacuated to vacuum). Without the axial bearing, the force would be difficult to overcome when rotating the turret by hand due to friction. Therefore, the axial deformation is limited by design to 0.25 ± 0.02 mm, as shown in Fig. 7.

In order to provide a safety mechanism for the turret to stay in place during vacuum evacuation, transport, or while in storage, four brass keepers are located around the perimeter to prevent accidental detachment. Full disassembly of the turret for maintenance and cleaning is possible after these parts are removed.

The outer rim of the turret is serrated for better hand grip while operating the device. Each sample position is marked by a notch used for indexing purposes. To switch the sample, the user simply has to press the lever to lift the latch mechanism (Fig. 8),

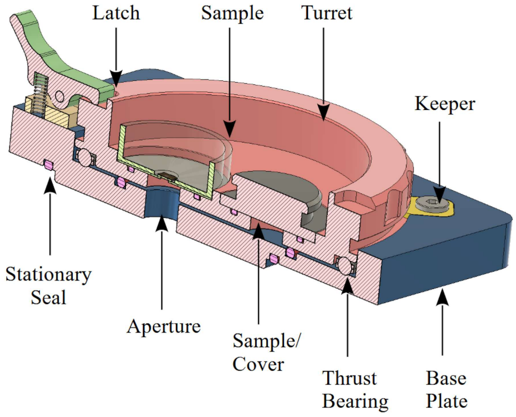

Fig. 6. Cross-section of the turret mechanism.

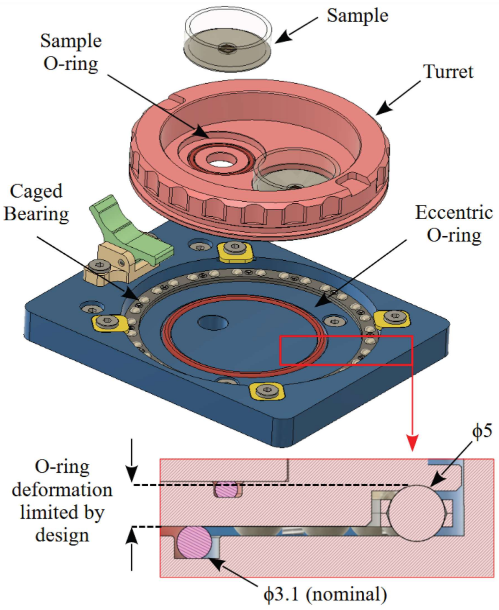

Fig. 7. Features of the turret system.

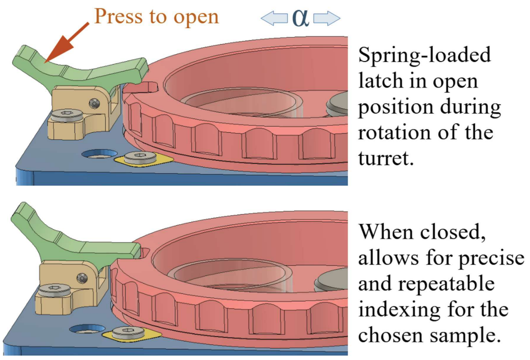

Fig. 8. Indexing latch mechanism.

rotate the turret to the desired position, and let go of the lever before the end of the rotation so that the mechanism can latch onto the desired position.

2.2.3. Turret operation using rotating face seal

In a face seal-type operation, an O-ring requires low roughness surfaces ($Ra = 1.6$ μm or less) to seal properly. Higher roughness could result in abrasive action against the O-ring, thus accelerating wear [14, 15]. In the case of the turret system, the eccentric O-ring (G-60) is standard, however, it is mounted eccentrically to the rotation axis of the turret by a small amount (3 mm), maintaining its circular shape. The idea behind this unusual approach is to provide (in conjunction with grease) a self-lubricating feature to the seal. While rotating, the grease would be constantly spread, creating a uniformly lubricated zone, as illustrated in Fig. 9.

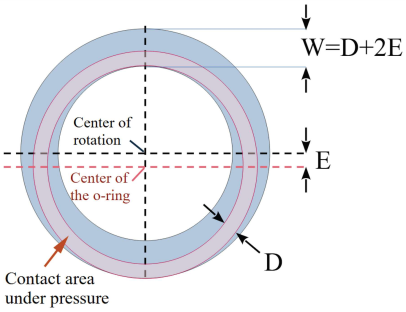

Fig. 9. Eccentric O-ring as a self-lubricating rotating face seal. Spread application zone (blue) is formed by the eccentricity of the O-ring (red). Width W is a function of the O-ring's contact area under pressure and the offset E.

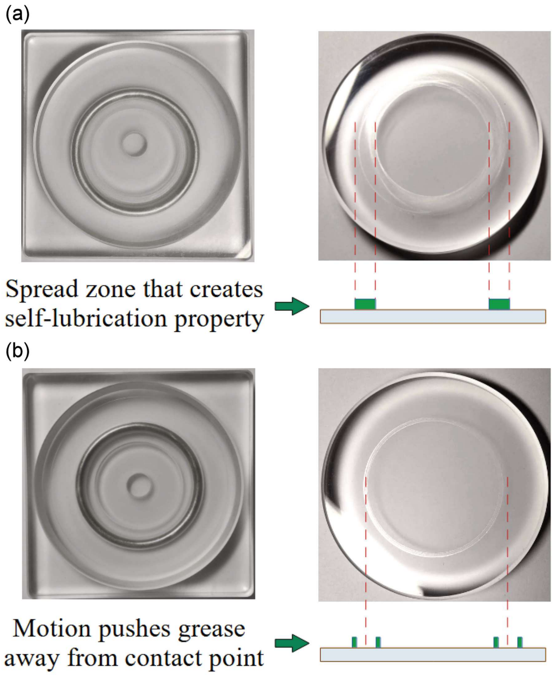

Fig. 10. Test jig for evaluation of the eccentric O-ring rotating face seal idea showing the spread application zone (a) compared with typical concentric O-ring approach (b).

The feasibility of the idea has been evaluated by creating a transparent jig that allows for visual inspection of the formation of the spread application zone of the grease (Fig. 10a). The concept proves to be indeed self-lubricating as opposed to using a concentric seal (Fig. 10b) where the grease is pushed to the sides, effectively preventing a significant friction reduction.

Lubrication of O-rings [16] in a vacuum system requires low-outgassing grease to prevent contamination of crucial components of the EB emitter, beam control systems, vacuum column, etc. The grease used in the sample switching system was JEOL Vacuum Grease 5G P/N: 781109736.

3. Results and discussion

The first test system to utilize the fast switching mechanism of the samples has been implemented as a 2-position system. It utilizes the smallest possible turret size for a given bearing/seal arrangement for a standard 35 mm culture dish. The goal is to test the required performance with minimal potential sources of vacuum leaks during testing. Two samples at 180 deg require half rotation of the turret to accomplish the position change. This allows for monitoring the pressure gauge for pressure fluctuations at the maximum expected angular step. Figure 11 shows the test turret system installed on the EB irradiation device.

The testing has been conducted by recording the stabilized starting pressure inside the vacuum column (for purposes of this experiment at 10^{-4} Pa), rotating the turret *clockwise* (CW) to the 2 position, and recording the pressure until it returned to the starting point. Subsequently, the test was repeated *counterclockwise* (CCW). Both tests were conducted at two rotational speeds, mimicking the typical range of use when operating by hand. Instead of sample chambers, stainless steel covers have been used. During operation, the eccentric seal provides a uniform and smooth operation, as expected. The system can be operated by hand with minimum effort, with CW and CCW operations showing similar characteristics. Figure 12 shows the test results.

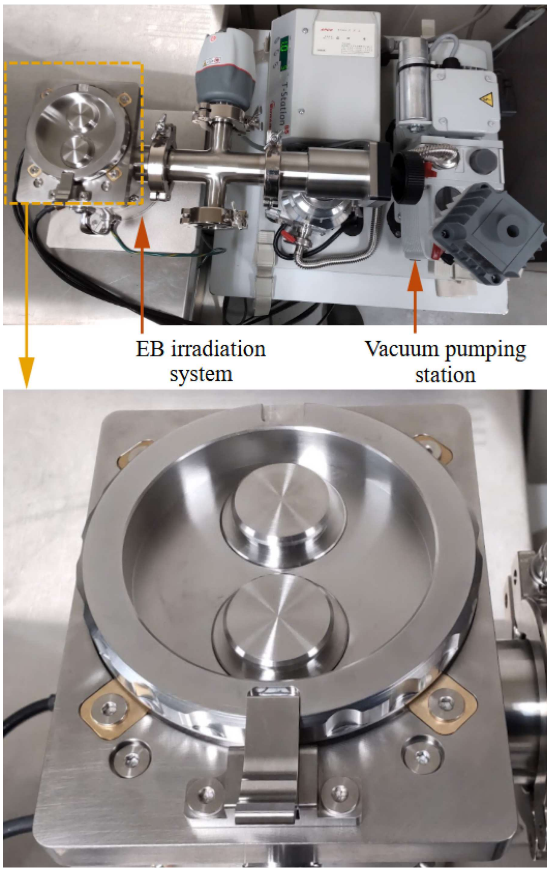

Fig. 11. Experimental 2-position fast sample switching turret assembly. Shown with two ∅35 mm covers installed.

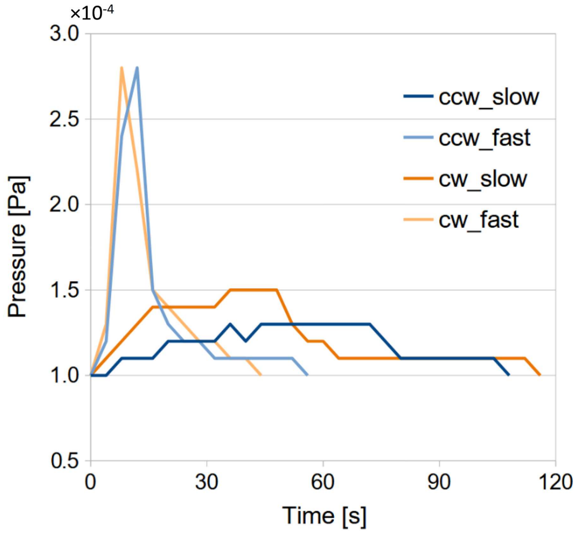

Fig. 12. Pressure fluctuation while changing position to another sample.

At the highest speed (∼ 15 s per position), the pressure fluctuation (green and yellow lines) has increased to the level of 2.8×10^{-4} Pa and returned to the initial value after roughly 45 s. At slower operation (45 s per position), the pressure increase is lower (max 1.5×10^{-4} Pa), however, it takes longer to stabilize, i.e., 80–120 s. The stabilization period is caused by the redistribution of the grease due to its surface tension, as well as O-ring material relaxation from brief deformation during the rotation due to eccentricity. It is important to note that the pressure fluctuation in both cases is much lower than the typical operational pressure of ASEM or EB irradiation systems (typically lower than 5×10^{-4} Pa), therefore the fast sample switching mechanism can be applied successfully to both. Components exposed to vacuum are made from SUS303 stainless steel.

4. Conclusions

The system presented in this paper is a significant step forward in increasing the productivity of ASEM and EB irradiation systems. With each additional sample loaded onto the turret-based fast-switching system, approximately 1–2 h can be saved on vacuum evacuation and re-pressurizing of the EB gun column. In the case of fast-drying live specimens, time savings are crucial for the efficient preparation of the experiments. The proposed self-lubricating rotary face seal based on an eccentrically placed O-ring allows for maintenance-free operation over long periods of time. The usage of vacuum-compatible grease provides a smooth hand operation of the turret as well as increased vacuum seal capability, as demonstrated by minimal fluctuation of pressure during operation. The scalable nature of the proposed system will allow for subsequent optimization of the mechanism and a further increase in sample amount capability.

Acknowledgments

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