

## **ATMOSPHERIC SCANNING TRANSMISSION ELECTRON MICROSCOPE**

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### **TECHNICAL FIELD**

This invention relates to the field of electron microscopy and in particular to environmental and atmospheric electron microscopy

### **BACKGROUND ART**

The electron microscope employs an electron beam generated in a vacuum envelope. The electron beam impinges on a specimen under examination and the emanated signals from the beam-specimen interaction are detected, then amplified and used to create an image of the specimen by well-established means. Conventionally, the specimen is also in the vacuum condition of the electron beam, which limits the range of possible applications of the generally high power, high magnification microscope. However, the advent of the later art of atmospheric and environmental electron microscopes has made it possible to examine specimens in a gaseous environment at any pressure between vacuum and one atmosphere.

In particular, some of the earliest attempts to view specimens in a gaseous environment are described by [1] who used a capsule to sandwich a wet specimen between two electron transparent films inside a transmission electron microscope (TEM). Because of the high rate of film breakage, [2] replaced the films with open small apertures and differential pumping to allow the examination of wet specimens also in the transmission electron microscope. Danilatos G D [3, 4, 5] further developed new detection methods and explored the differential pumping in their application to another form of electron microscope, namely, the environmental and atmospheric scanning electron microscope (ESEM and ASEM respectively).

The transmission form of an electron microscope (TEM) generally involves the use of a high accelerating voltage electron beam in the range of 100-1000 kV for the examination of generally thin specimens or sections of specimens through which the beam penetrates to yield information therefrom. In distinction, the scanning electron microscope (SEM) generally involves the use of an electron beam at low accelerating voltage in the range of 1-50 kV for the examination of the surface of bulk specimens. Those skilled in the art of electron microscopy should appreciate the complex nature of the two distinct TEM and SEM technologies and the different requirements and modes of imaging in these two branches of electron microscopy. Techniques used in one of these modes are not obvious that are applicable to the other, or if they do, there is no obviousness in what quantitative and qualitative relationship the various parameters should be involved.

The scanning transmission electron microscope (STEM) also operates in vacuum and traditionally involves accelerating voltages in the range 100-300 kV. One special form of STEM is described by [6] with pre- and post-specimen lenses. If the electron probe is scanned across the specimen plane by

means of scanning coils, which rock the electron beam around a pivot point at the front focal plane, then the scanned beam converges again to a pivot point behind the specimen at the back focal point, if the two planes are made conjugate.

Another similar STEM configuration proposed by [7] and also operating in vacuum provides an electron source, specimen and detector, all placed at conjugate planes of the pre- and post-specimen lenses. If a first set of scan coils is used before the specimen to scan the electron beam in a raster form at the specimen plane and if a second set of scan coils is used after the specimen in a synchronous fashion in order to de-scan or cancel out the scanning of the first set, then the beam again converges and focuses at a stationary point where detection takes place. This is referred to as confocal scanning electron microscope and claims to produce an optimum image contrast.

#### OBJECT OF THE INVENTION

The prime objective of this invention is to adapt the more recent techniques of ESEM and ASEM to the older attempts of introducing a gas to the TEM and to the more specific type of transmission scanning electron microscope (STEM). This objective cannot be simply achieved by the mere combination of prior arts without regard to serious problems and requirements emerging from the introduction of a gaseous environment inclusive of a full ambient atmosphere in the STEM. Only proper understanding of the interplay of various parameters can result in the successful design and operation of a novel atmospheric scanning transmission electron microscope (ASTEM) that for the first time will qualify as an industrial instrument. The novel aspects, inventive steps and commercial value of the ASTEM will become apparent in the description that follows.

#### DISCLOSURE OF THE INVENTION

The embodiments of the present invention relate to an atmospheric scanning transmission electron microscope (ASTEM), which is an expansion of the environmental and atmospheric scanning electron microscopes (ASEM and ESEM). Whereas the ASEM and ESEM employ an electron beam that scans the surface of bulk specimens inside an atmosphere or gaseous environment, the ASTEM employs an electron beam that scans and penetrates through the body of thin specimens enveloped by a gaseous environment or an ambient atmosphere. Whereas the signals emitted in an ASEM and ESEM originate from the near surface layer of bulk specimens and the detection of said signals generally occurs above the specimen under examination, the signals in ASTEM originate generally from inside the specimen and detectors are generally placed below the specimen while additional signals can still be detected from above the specimen. The ASTEM employs electron beams with accelerating voltage in the range 1-1000 kV or part thereof, so that it can be used both for the examination of the surface of bulky specimens at the low values of accelerating voltage as well as the inside of thin specimens penetrated at the high values of accelerating voltage.

In one aspect of the invention there is disclosed a device for the generation, propagation and focussing of an electron beam by the use of known art of an electron gun, beam acceleration, scanning

of beam and electromagnetic focussing of beam to a very fine electron probe that impinges on a specimen under examination. The generation and propagation of the said electron beam takes place in the high vacuum of an electron optics column. The high vacuum region of the electron optics column is separated from the high pressure of the specimen chamber via an intermediate pressure region by the use of differential pumping as employed in prior art of ESEM. The said intermediate pressure region communicates with the high vacuum and high pressure regions via two pressure limiting apertures respectively, through which the electron beam passes on its path towards the specimen. After the electron beam penetrates a thin specimen, there are, among others, two kinds of signal emerging from the other side of the specimen in the vacuum of a conventional STEM, namely, the bright field and the dark field signal resulting in either bright or dark field imaging techniques. The conventional detection techniques of bright and dark field imaging are not applicable because the specimen is in a gaseous environment which strongly scatters the electrons and merges the signals of bright and dark field which tend to cancel the contrast of each other, thus rendering imaging impractical or even impossible. It is the object of the present invention to disclose means for the separation and detection of the signals corresponding to bright and dark field imaging in the presence of gas at any pressure up to one atmosphere. The latter object is achieved by employing two concentric disk electrodes with a central orifice each aligned along the axis of the electron optical system and set at a specified distance apart. A third electrode in the shape of syringe needle, or fine metal tube, is also aligned coaxially on the axis of the optical system and inserted through the orifices of the first two electrodes. Each of the said three electrodes can move along the axis of the system, below the specimen, so that the distance between the disk electrodes and the position of the tubular electrode relative to the disk electrodes can vary. The specimen is held by a specimen stage and is placed between the final pressure limiting aperture and the first disk electrode. Thus, the entire sequence of the electron beam path involves first the extraction of electrons from the electron source, then a cooperative acceleration and focussing as the electron beam moves towards the first pressure limiting aperture of the intermediate pressure stage, then through the second (final) pressure limiting aperture into the high pressure specimen chamber, then penetration of the specimen and exit of the electrons from the other end of the specimen in the form of bright and dark field signals (electrons) carrying information from the bulk of the specimen. The bright field electrons are collected (trapped) in the cavity of the central needle electrode, whilst the dark field electrons having a higher scattering angle pass through the orifice of the first electrode below the specimen and interact with the gas molecules in the region defined between the first and second disk electrodes. The dark field electrons have high energy of the same order of magnitude as the accelerated electrons in the original beam and they dissipate their energy in the collisions they undergo with the gas molecules. Those electrons that may reach the second disk electrode or other nearby solid walls are generally backscattered in the gas and dissipate their energy in further collisions until they exhaust all their energy. During the dissipation of energy of the dark field electrons they ionise and excite the gas molecules producing negative ions, free electrons and photons to which we refer as the products of interaction of electrons with the gas. The two disk electrodes biased with an appropriate voltage detect the ions and

electrons. The photons are detected by nearby photon sensitive detector according to known art. The detected signals carry information about the specimen and this information is displayed or stored sequentially on an image or electronic file made in synchronisation with the fine electron beam spot scanned in a raster form through the specimen.

In another aspect of the invention the electrode bias voltage between the two disk electrodes is variable. Initially a low voltage of the order of a few tens of volts is sufficient to produce satisfactory imaging because the high energy dark field electrons produce a large number of electron-ion pairs thus creating a strong initial pre-amplification of the signal. The latter is the simplest condition under which the system operates, like an ionisation chamber. Further increase of the bias voltage, up to a few hundred volts, imparts additional energy to the freed gaseous electrons, which collide again with gas creating new electron-ion pairs in an avalanche amplification process that further amplifies the detected signal. The latter condition is known as a proportional counter and has been used in prior art of ESEM for the amplification of the low energy secondary electrons originating at the surface of the specimen. The use of principles pertaining to the ionisation and proportional counters simultaneously depends on the choice of accelerating voltage and pressure used in an ASTEM as these parameters vary the relative proportion of electrical charge produced in the gas between the detecting electrodes, whilst the total electrical charge is limited by the breakdown discharge condition, beyond which the detectors become inoperable.

In another aspect of the invention, the relative bias between the two disk electrodes and the tubular electrode is such as to prevent the low energy secondary electrons generated below and near the surface of the specimen to contribute in the detection of the dark field signal.

In another aspect of the invention, the distance between the disk electrodes is adjustable to optimise the amplification of the detected signal according to the choice of gas pressure and accelerating voltage.

In another aspect of the invention, the central tubular electrode is positioned at an appropriate point along the electron optical axis to optimise the bright and dark signal detection according to the choice of gas pressure and accelerating voltage.

In another aspect of the invention and to assist the separation of signals, an ancillary electrode with appropriate bias is placed in the space below specimen and between the specimen and first disk electrode. Such an ancillary electrode collects the secondary electrons that carry information about the bottom surface of the specimen. This signal may be used separately or discarded depending on whether the bottom surface of the specimen system is of interest or not, as will become clear during further description of the specimen presentation.

In another aspect of the invention a second ancillary electrode is placed above the specimen and between the top surface of the specimen and the nearest pressure-limiting aperture separating the specimen chamber from the intermediate pressure chamber. This electrode is biased with an appropriate voltage to determine the type and intensity of detected signal as both secondary and

backscattered electrons emerge in the direction above the specimen. The value of electrode bias is chosen according to the gas pressure and accelerating voltage used.

In another aspect of the invention an appropriate bias is applied to the grid incorporating the pressure-limiting aperture nearest to the top surface of specimen, in order to further assist in the detection of the secondary and backscattered electrons emerging above the specimen.

In another aspect of the invention an appropriate bias is applied to the grid incorporating the pressure-limiting aperture between the intermediate pressure chamber and the high vacuum column, in order to further assist in the detection of the secondary and backscattered electrons emerging above the specimen

In yet a further aspect of the invention an additional ancillary electrode biased with an appropriate voltage is placed between the two pressure limiting apertures, in order to further assist in the detection of the secondary and backscattered electrons emerging above the specimen.

In the preferred embodiment of the invention, scan coils are placed between the two pressure-limiting aperture to obtain a wide field of view in accordance with Danilatos prior art [5].

In another aspect of the invention x-ray detectors and cathodoluminescence detectors are placed either below or above the specimen in order to detect additional signals created at the specimen by the impinging electron beam in addition to or in lieu of the previously said photon detectors.

In yet a further aspect of the invention, the gas pressure in the specimen chamber varies from high vacuum to a full atmospheric pressure by the use of appropriate pumping and pressure regulating means. At the low pressure or low vacuum range of operation, the ASTEM allows the use of electron optics and detectors akin to the conventional operation of a STEM in vacuum. In order to accommodate a seamless transition from high pressure to high vacuum and vice versa, the present invention allows also the incorporation of prior art means of electron optics and detectors in the following embodiments:

In a further embodiment of the present invention, the incorporation of conventional means includes the condition that the scan coils are placed so that the apparent rocking (or pivot) point of the scanned electron beam is at the front focal point of the condenser lens of the electron optical system. An additional condition is the inclusion of a post specimen lens placed so that its focal point (known as back focal point) is conjugate to the front focal point of the condenser lens according to [6]. Under such conditions, the scanned beam re-emerges at the back focal point without the need of a second pair of scanning coils to de-scan the beam. By such techniques the separation and detection of the bright field signal is optimised giving rise to best contrast and resolution on the image. Those skilled in the art of conventional transmission electron microscopy should appreciate the details and workings of such prior art operating in vacuum conditions and no further details will be provided herewith.

In another embodiment of the present invention, the incorporation of prior vacuum art means includes an electron source, a specimen and a detector all placed at the conjugate planes of the pre-specimen and post-specimen lens, which optimises the collection and detection of the bright field signal. In the scanning mode, there are two sets of scanning coils with a first set scanning the electron beam in a raster form through the specimen and with a second set of coils de-scanning the emerging bright field

to a stationary point. By such means the contrast and resolution are optimised and no further details will be provided herewith, as the skilled person in this field can appreciate.

Attention will now be drawn to various forms of specimen and specimen presentation in the device of the present invention. In one such form, the specimen is of sufficiently small thickness so as the given accelerating voltage to allow the electron beam to penetrate through it and produce the bright and dark field signals at the other end of it. In this form, the specimen rests on a fine mesh grid allowing support of the specimen while at the same time it is free on both upper and lower side over the free spaces of the mesh. Should the specimen be smaller than the mesh spacing, a very fine electron transparent film is first deposited on the mesh, according to know art, whereupon the specimen can freely rest. In the latter case, the secondary electrons emitted from the bottom of the film carry no information from the specimen of interest and can be separated out and discarded by appropriate choice of bias on the electrodes disclosed in the present invention.

In another form of specimen presentation the present invention allows the use of a fine mesh grid with a electron transparent film as previously said whereupon the specimen is inside a liquid medium, such as living specimens in vitro. By operating the ASTEM at ambient pressure, the specimen is unaffected by otherwise pressure variations, and thus the un-disturbed specimen can be brought under the electron beam for imaging. This is a case where dark field and backscattered electrons are of main interest and can be separated out and detected by the detection means disclosed in the present invention.

In yet another form of specimen presentation, the specimen is encapsulated in a liquid form between two electron transparent films in a manner similar to prior art [1]. Whereas such method was used in prior art under high vacuum with concomitant high chance of film breakage and risk of disruption of operation or even instrument damage, the present invention allows such use without the risk of disruption and instrument damage, because the ASTEM can tolerate high pressures. Thus, the encapsulated specimen is now used both in high vacuum and preferably at low vacuum or low pressure that still allow particular detection modes and imaging to be used. The latter possibilities include the combination of wet specimen examination together with preferred conventional techniques giving rise to novel industrial possibilities, none of which was obvious or was practised by any of the prior arts.

In yet a further form of specimen presentation, the specimen is placed inside an environmental cell, the space of which is confined between two single hole aperture grids held apart by a spacer wall. The space inside the cell is connected to ambient conditions via pressure regulating means, according to prior art [2]. Thus the environmental cell provides its own two pressure limiting apertures that allow the specimen preferably to be at ambient pressure but also it allows the choice of lower pressure environments with any choice of gas. The specimen can then be placed inside the cell either supported by a grid or in combination by a grid/film system as previously described. It should be appreciated that that this adds to the complexity of operation of the system but it provides a novel approach that expands the purpose of the present invention and surpasses all prior art.

It will be apparent to those skilled in the art that various changes and modifications may be made therein to allow a combination and integration with other instruments without departing from the spirit or scope of the invention. Such ancillary technologies include, but not limited to, image analysis and manipulation techniques, computational techniques, stereo-micrographic imaging and three-dimensional re-constructions.

Parts of the disclosed invention should not be considered as constituting a separate device, such as, for example, the operation of the upper part of ASTEM used as an ASEM. In the latter case, the specimen chamber can be eliminated altogether, the accelerating voltage be chosen at very low value with subsequent design of a small and portable electron optical column. The latter ASEM can be portable or be fitted with removable specimen chambers.

These and further objectives of the invention will become apparent from the following description of the preferred embodiments of the present invention as illustrated in the accompanying drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagram of the ASTEM showing detection configurations in relation to the specimen, gaseous specimen chamber and electron optics.

Fig. 2 is a specimen freely deposited on grid or on thin film supported by grid.

Fig. 3 is a specimen inside a liquid film resting on a support grid or on film and grid.

Fig. 4 is a specimen in sealed capsule made by two film and two grid systems spaced apart by a ring spacer.

Fig. 5 is specimen in an environmental cell having two coaxial pressure limiting apertures with gas pressure regulation provided via a side port opening.

#### BEST MODES FOR CARRYING OUT THE INVENTION

To assist with understanding of the invention, reference will now be made to the accompanying drawings, which embody some examples of the invention.

One embodiment of the device of the present invention is shown in Fig. 1. An electron beam 1 is generated by an electron gun (not shown) and accelerated and propagated by known electron optical means (not shown). Said beam travels along the axis of the electron optics, strikes and penetrates through a thin specimen 20, with which interacts and produces a variety of signals shown by dotted lines, namely, high energy transmitted electrons 2 close to the axis of the system, high energy transmitted electrons 3 further away from the axis, low energy electrons or otherwise called secondary electrons 4 emitted from the lower surface of the specimen 20, high energy backscattered electrons 5 emitted in the direction above the specimen, low energy or secondary electrons 6 emitted from the upper surface of the specimen 20. The transmitted electrons 2 are associated with the bright field image, whilst transmitted electrons 3 are associated with the dark field image. In this first embodiment, the electron beam 1 is focussed to a very fine electron probe via a system of electromagnetic lenses 7 placed in the space before the beam strikes the specimen (pre-specimen location). A set of scan coils 8 causes the electron

beam 1 to scan the specimen in a raster form according to known art. The specimen is placed on a specimen stage 9, which allows movement of the specimen in all primary directions X, Y and Z. The upper portion of the electron optics column space 30 is maintained at high vacuum by known techniques but is allowed to communicate with the lower part of the column space 31 via a small orifice 50, also referred to as pressure limiting aperture (first aperture). Said space 31 also communicates with specimen chamber space 32 via a small orifice 51 also referred to as pressure limiting aperture (final aperture), and is connected with pumping means 33 to pump gas out via port 34 and to maintain low or good vacuum in said space 31. Specimen chamber 32 is maintained at any pressure between vacuum and one atmosphere via port 35 and pressure regulating means 36. By this embodiment of the invention it is possible to maintain a high pressure in the specimen chamber, while the small amount of fugitive gas via aperture 51 is pumped out of the intermediate pressure stage 31 and only a negligible fugitive amount of gas leaks through aperture 50, which allows the maintenance of high vacuum in the upper column space 30. A first disk electrode 42 with a central orifice 52 and a second disk electrode 43 with a central orifice 53 are coaxially placed below the specimen and set apart by a given distance 10. These electrodes are biased with a variable voltage 62 and 63 so that the potential difference between them is chosen to suit different imaging conditions. A third tubular electrode 44, syringe needle like, with a small diameter and hollow cavity 54 is biased with voltage 64 and is inserted through the orifice of previous disk electrodes along the axis of the system so that its tip can be placed close to the bottom surface of the specimen. By such electrode configuration, the bright field electrons 2 are captured and trapped inside cavity 54. The dark field electrons 3 are collected by the disk electrodes 42 and 43 in the space between them. In the presence of gas in the specimen chamber, the dark field electrons 3 collide with the gas molecules creating free electrons and ions 11 as well as photons 12 by excitation of the gas molecules. The number of electron-ion pairs and photons is very large at high chamber pressure creating a strong initial amplification of the dark field signal. This pre-amplified electrical current signal is picked up by either electrode 42 or 43 and by subsequent electronic process is used to produce an image according to known techniques. The secondary electrons from the bottom of the specimen are either repelled or collected by electrode 42 through appropriate choice of bias. Because the dark field signal 3 is initially amplified whilst signal 4 is not, the latter does not interfere with the image for all practical purposes. Suppression of this type of interference can be further facilitated by applying and choosing appropriate voltage on electrodes 42 and 44 so that all signal processes taking place in the space between specimen and electrode 42 can be separated out from the processes between electrodes 42 and 43 wherefrom the dark field image is formed.

In an improved embodiment of the invention, the potential difference between electrodes 42 and 43 is increased just before the point of inducing a breakdown discharge, so that the signal is further amplified proportionally by the well-known ionisation avalanche cascade 13.

In a further embodiment of the invention, the photons 12 created in the space between electrodes 42 and 43 are detected by any photo-sensitive means, such as photomultiplier or other means 14, and this signal is used to produce an image according to known techniques.



In a further embodiment of the invention, an ancillary electrode 47 biased with voltage 67 is placed between specimen 20 and electrode 42 in order to collect the secondary electrons 4 generated at the bottom surface of the specimen. Because the space between the specimen and electrode 42 is limited, electrode 47 is preferably a thin wire or a ring electrode coaxial with the axis of the system.

In yet a further embodiment of the invention, the secondary electrons emitted from the top surface of the specimen are collected by a thin wire or ring electrode 46 coaxially placed in the space between the specimen and aperture 51 and biased with voltage 66.

In another embodiment of the invention, the aperture grid 41 acts as a collection electrode biased with voltage 61. This electrode collects both secondary and backscattered electrons.

In another embodiment of the invention, a wire or ring electrode 45 biased with voltage 65 is placed between apertures 50 and 51 for the collection of either electrons 5 or electrons 6, depending on the gas pressure and the applied voltages on relevant electrodes.

In a further embodiment of the invention the aperture grid 40 biased with voltage 60 acts as a collection electrode for either electrons 5 or electrons 6 depending on the applied bias on relevant electrodes and prevailing pressures in the system.

Any or all of the biases 60, 61, 62, 63, 64, 65, 66 and 67 have either fixed values or variable positive and negative or ground potential values to increase the versatility of the device. In particular, the preferred bias of all electrodes above the specimen are biased with a voltage sufficient to create a gaseous ionisation avalanche and photon avalanche for the pre-amplification of the corresponding signals. For example, such an ionisation avalanche is shown by 15 and a photon avalanche by 16 in the region between electrodes 41 and 42, but similar effects can be produced in the space between specimen and aperture 51..

In a general embodiment of the invention, any number of electrodes, or a combination of electrodes 40, 41, 42, 43, 44, 45, 46 and 47 is used in accordance with application type of the device. Thus, any one electrode separately or in combination with others can be used as a detector for any signal or mixture of signals. Similarly, any one electrode or any combination of said electrodes can be used to filter out unwanted signals.

In another general embodiment of the invention, any number of photosensitive detectors or combination thereof is used in the spaces between 40 and 41, between 41 and specimen 20, between specimen 20 and 42, or between 42 and 43.

In any of the previous embodiments, the distance between electrodes 42 and 43, or the relative position of electrode 44 is variable.

Another embodiment of the invention operating at low vacuum or vacuum employs post specimen lens 17 for the purpose of collecting the bright field signal in accordance with conventional techniques known for vacuum STEM. In such embodiment, the post specimen lens is positioned relative to the pre-specimen lens so that their focal planes are conjugate. If the rocking (pivot) point of the incident beam is located at the front focal plane, then the scanned electron probe exits from the specimen and converges to a stationary point at the back focal point and no additional de-scan coils are required. This simple

approach provides an advantage for increasing the bright field contrast by collecting a maximum amount of bright field electrons by simple detection means.

Another further embodiment of the invention operating at low vacuum or vacuum employs a pre specimens lens 7 and post specimen lens 17 in such a way that the electron source, the specimen and an electron collector are all located at respective conjugate points for the purpose of operating the STEM in the so called confocal mode. In such embodiment, there is a second set of de-scanning coils 18, synchronised with the scanning coils 8, so that the scanned electron probe exits from the specimen and converges to a stationary point at the back focal point. This approach provides a further advantage for optimising the bright field contrast by collecting an optimum amount of bright field electrons by simple detection means.

All of the previous embodiments are facilitated by proper specimen presentation to the electron beam. The following drawings embody the best means for specimen insertion in the ASTEM:

Fig. 2 shows a specimen of small particles 21 resting on an electron transparent film 23 that is fixed on a very fine mesh grid 22. It is typical to use a very thin material film, which allows an electron beam to transmit with minimal electron collisions and minimal loss of beam intensity. This is usually attached onto a very fine metal mesh grid with free spaces to allow the passage of the electron probe. Should the specimen be flat and large enough, i.e. larger than the mesh spacing, such as a bio-polymer or other specimen, the use of support film 23 is not necessary and the specimen 21 is placed directly on the grid 22. The assembly of components in this drawing is used in lieu of item 20 in Fig. 1.

Fig 3 shows a diagram with a liquid layer 24 inside which particles, such as microbes or other, are located. This liquid layer can be self supporting on mesh grid 22 or supported by an electron transparent film at the bottom or both at the bottom and top of the liquid layer. The assembly of components in this drawing is used in lieu of item 20 in Fig. 1.

Fig. 4 shows a diagram of a capsule consisting of two metal mesh grids 22 and 25 held apart by a spacer holder 27 and two supported electron transparent films 23 and 26 each adhering to the inside face of the mesh grids. By such means a fine specimen 21 can be placed inside a liquid phase 24 maintained at ambient pressure, while the capsule is exposed to high or low vacuum in the ASTEM. The assembly of components in this drawing is used in lieu of item 20 in Fig. 1.

Fig. 5 shows a diagram of an environmental cell 80 with two coaxial pressure limiting apertures 81 and 82, and port 83 with pump and pressure regulating means 84. Inside the cell an object for examination is placed by itself or on supporting means as in Fig. 2 or Fig. 3 and the pressure is maintained either at ambient atmosphere or any other controlled environment. The assembly of components in this drawing is used in lieu of item 20 in Fig. 1.

It should be appreciated that Figs. 1, 2, 3, 4 and 5 do not restrict the scope and design of the present invention. Various parameters can vary to obtain a different information in different applications. It will be apparent to those skilled in the art that various changes and modifications may be made therein without departing from the spirit or scope of the invention.

The integration of pressure limiting apertures 50 and 51 with detecting electrodes 40 and 41 is shown as one preferred embodiment for compactness of design but the electrodes can be separated from the aperture grids. Conversely, the pressure limiting apertures can also be used as spray or probe forming apertures according to electron optics design in order to further improve the compactness of the device.

The specimen stage 9 can be constructed to include additional specimen movements such as rotation and tilt, while the electrodes 42, 43 and 44 are removable so as to allow conversion of the ASTEM to an ESEM or ASEM, which permit the introduction of large specimens for surface examination and analysis.

Again it should be appreciated that the drawings referred to herewith do not limit the scope of the present invention as other embodiments not shown in the diagrams are also within the disclosure of the invention. For example, x-ray detectors and cathodoluminescence detectors are placed either below or above the specimen in order to detect additional signals created at the specimen by the impinging electron beam in addition to or in lieu of the previously said photon detectors. The ASTEM can be used as ASEM, can be made portable with or without detachable specimen chambers. Ancillary external techniques can be used without departing from the spirit and scope of the general disclosures of the present invention.

#### INDUSTRIAL APPLICABILITY

The conventional SEM and STEM have been successful commercial instruments for many decades despite the great disadvantage that examination of specimens can only be done in vacuum conditions. As a result, their applications have been limited only to dry and conductive specimens, or dead organisms which have undergone severe preparation modifications prior to their examination. The introduction of a gaseous environment resulted in the ESEM technology with great industrial applicability. Any improvement or expansion of the ESEM should have similar and further industrial applicability.

The disclosure of the present invention describes a device that expands the ESEM to operate also in the transmission mode, and in the presence of any gaseous environment at any pressure up to an atmosphere. Therefore, the ASTEM is a universal electron microscope with practically unlimited applications.

The applications of ASTEM are in many fields of science and technology, research and development, and in other industrial applications.

It should be possible to succeed where many prior art attempts failed including the examination of live specimens in vitro.

By virtue of the novel aspects of the present invention there are numerous advantages over prior art. One advantage is the possibility to examine the inside of thin specimens or thin sections of specimens in their natural state, wet or dry, insulating or conductive, live or dead.

Another advantage is the use of thickness of specimens that is much greater than the thickness used by conventional means.

Another advantage is that whole biological specimens with thickness of the order of 10 microns are now possible.

Another advantage the universal use of the ASTEM as it can operate seamlessly from vacuum to atmosphere incorporating all types of imaging techniques.

## REFERENCES

To be listed during final specification.

## CLAIMS

To be listed during final specifications

## ABSTRACT

The invention provides for an atmospheric scanning transmission electron microscope that allows the examination of thin specimens inside a gaseous environment up to an atmosphere pressure. The electron beam after transmitting through the specimen produces bright and dark field electrons, which interact with the gaseous atmosphere to produce electron-ion pairs and photons. A system of suitable biased electrodes collect the bright and dark field signals amplified by the gaseous medium. A photon detector collects the light produced by the dark field electron in the gas. By such detection means, images are constructed carrying information from the bulk of the specimen, which includes live biological specimens. The electron optics and position of scanning coils are configured to produce image optimum contrast. The device further allows for operation as an environmental or atmospheric scanning electron microscope as well as under vacuum conditions, giving rise to a universal electron microscope.

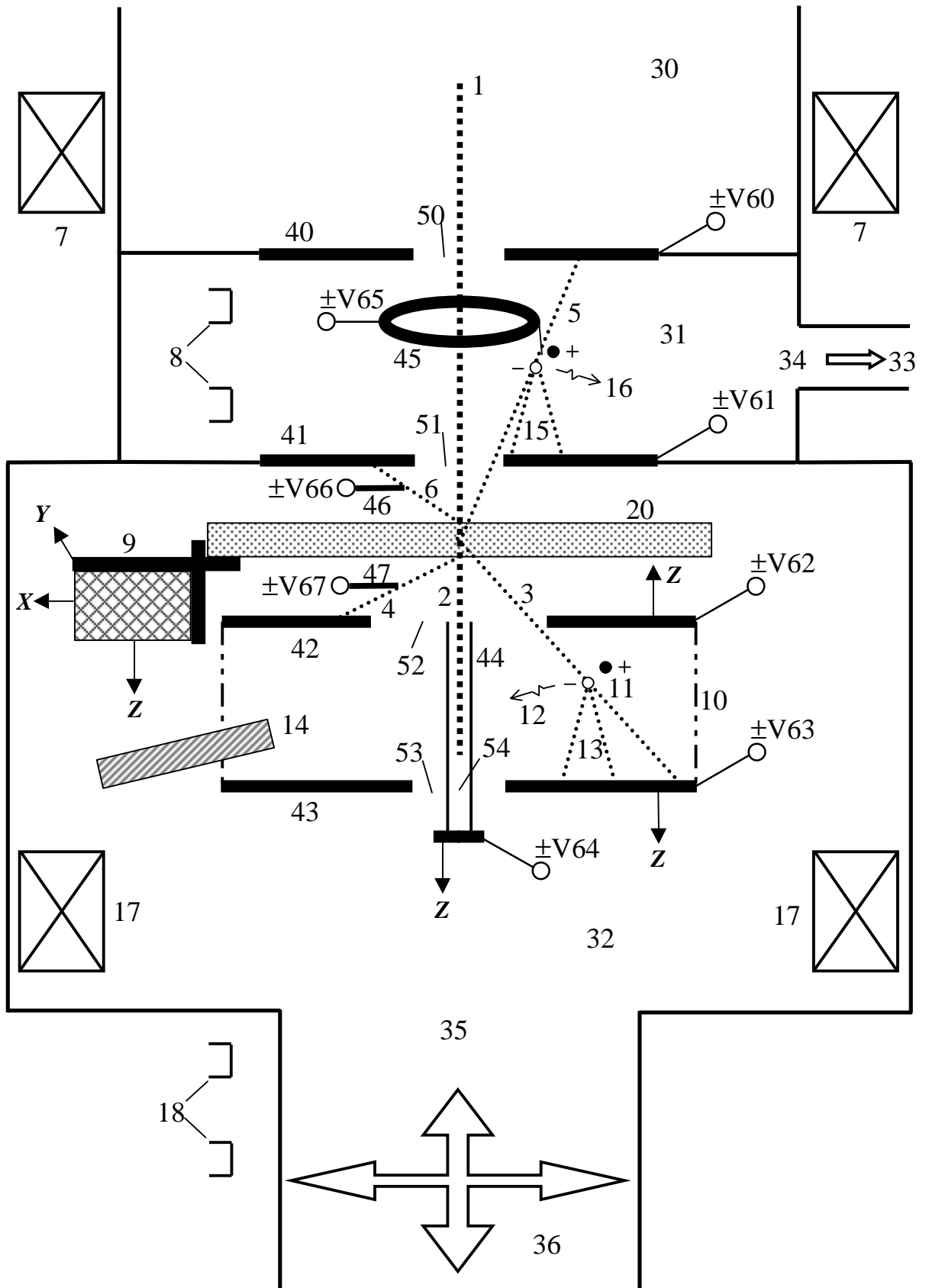


FIG 1

