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(71) **Applicant: VILNIAUS GEDIMINO TECHNIKOS UNIVERSITETAS** [LT/LT]; Saulėtekio al. 11, 10223 Vilnius (LT).

(72) **Inventors: BUČINSKAS, Vytautas**; S. Kairio g. 10, 10231 Vilnius (LT). **MORKVĖNAITĖ-VILKONČIENĖ, Inga**; Saulės g. 11-2, Platiniškės, 14171 Vilnius (LT). **DZEDZICKIS, Andrius**; Nendrių g. 3A, 13107 Vilnius (LT).

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(54) **Title:** SYSTEM AND METHOD OF DETERMINING THE DIMENSIONS OF A SPATIAL MICROMETRE-SIZED OBJECT AND ITS SURFACE RELIEF

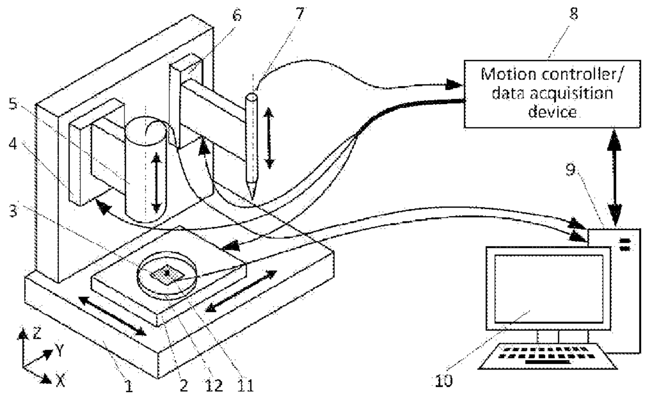

Fig.1a

(57) **Abstract:** The invention is intended for determining the dimensions and surface relief of micrometre-sized spatial objects by generating a composite image of the examined spatial object using the microscope. The invention aims to improve the detection quality when object is transparent or partially transparent or when the surface of the object is soft and/or easily damaged. The examined three-dimensional micrometre- sized object is photographed from above with a microscope, and the contour of the micrometre-sized object is determined by the sensor with an electrode for measuring electrochemical properties between the sensitive surface of the mentioned electrode and the surface of the micrometre-sized object at each scanning point, thus determining the height of object under investigation and its coordinates at each point at the scanned point. Thanks to the program installed in the computer processor, a general image of the examined spatial micrometre-sized object with its real dimensions and its surface relief is obtained.

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System and method of determining the dimensions of a spatial micrometre-sized object and its surface relief

Technical field to which invention relates

The invention relates to systems and methods for determining the dimensions and surface relief of spatial objects of micrometric size. The micrometre-sized spatial objects under examination can be biological objects, for example, human, animal, or unicellular tissue cells. The study of such micrometre-sized objects is necessary for practical and scientific research, and operation in medicine, biology, biochemistry, and related fields. The study of such micrometre-sized objects faces the problem that the optical image quality is limited when examining micrometre-sized spatial objects with dimensions close to the wavelength of light. On the other hand, vibrations of mechanical systems, and radial and axial clearances in gears interfere with the operation of the optical microscope.

The invention is intended for a complex solution in the examination of spatial micro-objects when the object's center, area, relief, and electrochemical properties are determined by the proposed device using the developed system using a research algorithm based on sensor data fusion.

Indication of the background art

Many methods and devices are currently used for the visualization of micrometre-sized objects. One such device is described in US patent US7391565B2, which describes an optical scanning device for collimated focus multi-optical systems. It has a built-in optical system, which is designed in such a way that the focus of the illuminating beam is concentrated on the point of illumination of the object, i.e., at the observation point. On the other hand, the focus of the light beam goes from the illuminated point to the illuminated point on the plane of the object; at least one rotatable mirror is provided, which reflects the illumination beam while allowing scanning of the observed object concerning the observation plane. At the same time, the light beam projects a light spot in a fixed position. A first spatial light filtering system is mounted on a first object plane that filters a light spot that represents the position of the light beam. Such a system is very advanced and efficient but expensive, requires special protection against external vibrations, and is suitable only for objects with a pronounced flat surface.

Another known device for visualizing micrometre-sized objects is described in the German patent DE102011000835B4. It features a scanning microscope with a built-in light unit that emits a beam of light that passes through the lens, concentrates the light beam on the object under examination, and the scanning device moves the focus of illumination around it. The direction of the beam is changed by aligning the light beam with the eyepiece of the objective. This type of optical scanning microscope ensures high image quality, but it is also sensitive to the background of environmental vibrations and the light absorption of the material of the object being examined. The device is also expensive, has overlapping optical systems, and therefore requires periodic adjustment.

Another known device for visualizing micrometre-sized objects includes the adaptive scanning microscope described in Canadian patent CA2887052C, in which the projected light beam is positioned along more than one axis by rotating the light beam source or optical guidance system. This optical system is very compact, significantly surpassing the characteristics of the lens and mirror system of the classical configuration. The adaptive optical system has very low dispersion, chromatic aberration, and peripheral aberration. This system has excellent optical properties, but the actual zooming of the object becomes a problem since the magnification of the optical system varies with the distance to the object, which depends on the brightness. Furthermore, this system is of high price.

Japanese patent application JP2019056917A describes a method and apparatus for visualizing micrometre-sized objects with fully automatic rapid scanning and digitization of a microscopic specimen. The proposed device has an electric drive: the first solution is when the sample is pushed at a constant speed on the surface of the sample along the main axis from the defined first position to the defined second position, the second solution – is when the sample moves along the surface of the sample along the second axis, perpendicular to the main axis of movement. The scanner is equipped with an electric drive; a lighting system; a camera mounted perpendicular to the surface of the specimen and the main axis; data processing equipment with a processor for image data processing and storage. The specimen is first moved along one axis and then moved along a second axis, thereby ensuring a complete scan of the specimen. Such a scanning system is efficient, but expensive and only uses data from one camera to evaluate the image of the specimen, resulting in an image with insufficient sharpness.

A device for visualizing micrometre-sized objects is described in US patent US10725279B2, which uses an advanced optical microscope. It consists of a micro-monitor that acts as a matrix of light sources, creating structured specimen illumination, an image sensor that captures an image of the specimen, and a microscope lens that directs the light beam from the illuminating matrix toward the specimen and the reflected light to the image sensor. The microscope objective has a changeable focal length, and its change on the optical axis strongly changes the magnification of one of the illuminated points falling on it, the image of which is recorded on the image sensor. This device only works with optically detectable terrain using reflected light, so it is limited in working with micrometre-sized biological objects as they are mostly transparent.

PCT patent application WO2021196419A1 describes a device for visualizing micrometre-sized objects in 3D format by recording the spatial coordinates of points on the surface of the object using a non-destructive optical technique, a particularly deep-field microscopic system, which is related to the non-destructive three-dimensional measurement of photoelectric cells. The microscope system consists of an optical microscope, a photoelectric contour sensor, a three-dimensional positioning device, a control module, and a PC processor, where the three-dimensional positioning device consists of a platform operating along the x-y axes and a separate z-axis lifting module. An optical microscope is matched with an illumination source and an imaging device. The control module includes the three-dimensional positioning device control unit, and the signal processing unit is connected to the photoelectric contour sensor. The system includes a power source, and the image recording and transmission unit transmits the received image to the computer. By applying the appropriate measurement method with the spatial ultra-deep-field microscope system, the specimen can be observed non-destructively, taking a 3D colour image and performing precise 3D measurement in a large field, achieving accurate object dimensions while maintaining the details and accurate colours of the object.

This method is difficult to apply to the examination of transparent biological objects because the image reflection when the beam of the light source is not perpendicular to the illuminated surface is of low quality and difficult to apply to image processing. Vibrations of mechanical systems and radial and axial clearances in drives interfere with the operation of the optical microscope.

European patent EP 3092459 B1 describes a method for creating a composite image of a three-dimensional object in the study of micrometre-sized objects using a microscope, where multiple overlays of three-dimensional images of different areas of the object are used with at least one image of an adjacent surface with sufficient image resolution to create a three-dimensional image of a microstructure with dimensions perpendicular to the lens and along the optical axis of ten micrometres or less. Each image with a separate coordinate system defines the position of the body and the normal direction of the surface fragment, the parameters of the overlapping fragments are included in the overall image. The image obtained of the microstructure is reconstructed according to the parameters of the overlapping regions, and the combined images form a common spatial image.

The known patent describes a system for creating a three-dimensional composite image of the surface of a micro-object under examination, the system includes a housing in which a microscope is mounted for spatial representation of the surface features of a micro-object under examination, a holder for positioning the micro-object under examination, a positioning devices for moving mentioned holder and the microscope longitudinally relative to each other along the x-axis and/or along the y-axis and/or along the z-axis to place the object under examination in a selected position relative to the optical axis of the microscope, a computer with a programmable processor communicating with the microscope and programmed to obtain images of the microstructures under examination with different coordinates and combine them, forming a general image of the studied spatial micro-object.

This method and system have solved the problem of illumination perpendicular to the surface, but this method is based on the use of reflected light to conduct research, so when studying transparent or partially transparent micro-objects or micro-objects with a soft and/or easily damaged surface, the obtained and displayed spatial image of the micro-object is of insufficient quality.

Technical problem to be solved

The invention aims to improve the quality of the spatial image of spatial micrometre-sized object and the determination of its surface relief when the object is transparent or partially transparent or when the surface of the object is soft and/or easily damaged.

Disclosure of the Invention

The essence of the solution to the problem according to the proposed invention is that in a system for determining dimensions of a examined spatial micrometre-sized object and its surface relief, by generating a composite image of the examined spatial micrometre-sized object, comprising: a housing in which a microscope is installed, a holder for placing the examined spatial micrometre-sized object, a positioning device to move the holder and the microscope relative to each other along the x-axis and/or along the y-axis and/or along the z-axis to place the examined spatial micrometre-sized object in a selected position relative to an optical axis of the microscope, a computer with a main programmable processor that communicates with the microscope and is programmed to obtain a general three-dimensional image of the examined spatial micrometre-sized object, wherein the system is provided with an optical sensor and an electrochemical properties measurement electrode, wherein the positioning device which is installed in the housing comprises:

- a horizontal positioning device with degrees of freedom along the x-y axes,
- a first vertical positioning means having a degree of freedom along the z-axis, and
- a second vertical positioning means having a degree of freedom along the z-axis and located in the housing at a selected horizontal distance from the first vertical positioning means, wherein

the optical sensor is attached to the horizontal positioning device, above which a holder is attached, in which the examined spatial micrometre-sized object is placed, mentioned microscope is attached to the first vertical positioning means, and the electrochemical properties measurement electrode is attached to the second vertical positioning means, wherein the horizontal positioning device is mounted with the possibility to move in the directions of the x-y axes and to be placed in selected positions in relation to vertical axes of the microscope and the electrochemical properties measurement electrode, wherein

during the operation of the system, when the examined spatial micrometre-sized object is in the field of view of the microscope it is photographed by the microscope and the real-size outline of the examined spatial micrometre-sized object is captured by the optical sensor, wherein data from the microscope and the optical sensor is transmited to the main processor of the computer and its memory;

and

during the operation of the system when the examined spatial micrometre-sized object is in the field of the measuring electrochemical properties electrode it is scaned out in the x-y plane defined by the contour of the spatial micrometre-sized object according to the trajectory, number of scanned points and their density set by the computer main processor and is measured electrochemical properties at each scanned point, which is associated with the real coordinates of the positioning means and at each scanned point and data is recorded in the memory of the computer,

the main processor of the computer is programmed in such a way that the aforementioned data recorded in its memory, received from the microscope, optical sensor, and from the electrochemical properties measurement electrode, the data of which is linked to the coordinates of the scanned points are merged by obtaining a total spatial image of the examined spatial micrometre-sized object with its real dimensions and its surface relief.

At the scanned point, the association of the electrochemical activity measured by the electrode for measuring electrochemical properties with the real coordinates of the positioning devices at each scanned point is obtained according to the control commands that are generated in the main processor, taking into account the size and shape of the spatial micrometre-sized under examination selected by the user, thus creating a trajectory for scanning electrochemical properties with the electrode.

The micrometre-sized object for examination can be selected from biological objects, such as humans, animals, or single-celled tissue cells.

The micrometre-sized object under examination may include one, several, or many of the cells.

Another example of the implementation of the proposed invention is a method of determining the dimensions of a examined spatial micrometre-sized object and its surface relief by generating a composite image of the examined spatial micrometre-sized object using a microscope , wherein the method comprises the following steps:

- taking an image of a top view of the examined spatial micrometre-sized object with a microscope and transferring the data to the memory of the computer (9),
- capturing a real-size contour of the examined spatial micrometre-sized object (11) with an optical sensor,
- moving the examined spatial micrometre-sized object into the measuring field of an electrochemical properties measurement electrode,
- scanning the examined spatial micrometre-sized object by electrochemical properties measurement electrode in the x-y plane defined by the examined spatial micrometre-sized object contour according to the trajectory and the set number of scanned points and their density set by the computer main processor, wherein at each scanned point the electrochemical properties measurement electrode measures the electrochemical activity, which determines the height of the examined spatial micrometre-sized object at the scanned point,
- linking the measured electrochemical activity at each point with the real coordinates of each point and transferring the obtained data to the main computer (9) memory,
- thanks to the program installed in the computer main processor merging of data obtained from the microscope, the optical sensor, and the data from the electrochemical properties measurement electrode, respectively associated with the coordinates of each scanned point, by obtaining a general image of the examined spatial micrometre-sized object with its real dimensions and its surface relief.

Advantages of the invention

The proposed system and method perform automated measurement of the surface projection (contour) of a micrometre-sized object and determination of surface relief. It automatically renders a true-dimension 3D (spatial) model of the examined spatial micrometre-sized object.

This invention makes it possible to create a high-quality image of a three-dimensional micrometre-sized object and obtain its real size because the image obtained by the optical microscope is supplemented with real-size parameters from the optical sensor, electrode for measuring electrochemical properties, and positioning devices. This invention makes it possible to determine the actual dimensions of an object regardless of its transparency.

This is achieved by using a microscope camera, an optical sensor, a positioning device using a stepper motor as a drive, the position of the output shaft of which is known by the number of control pulses given to the motor, and an electrode for measuring electrochemical properties mounted on a vertical axis for examining the surface of the object under examination by determining its height. The system for examining a micrometre-sized spatial object has a three-axis positioning device with an additional vertical axis for an electrode for measuring electrochemical properties, which allows determining the real size of the object under examination by supplementing the optical image with real coordinates calculated according to the given angles of rotation of the stepper motors of the positioning device.

Brief description of the drawings

Fig. 1a shows a schematic diagram of the system for determining the dimensions and surface relief of a micrometre-sized object in the first step when the microscope is over the object under examination. Fig. 1b shows the scheme of the system for determining the dimensions and surface relief of micrometre-sized objects in the second stage when the electrode for measuring electrochemical properties is above the object under examination.

Fig. 2 shows the system according to Fig. 1a and Fig. 1b logic diagrams. Fig. 3 shows the algorithm of the physical operation of the system.

Fig. 4 shows the logic diagram of the fusion of sensor signals. Fig. 5 shows a sample with clusters of micrometre-sized objects.

Examples of realization of the invention

The proposed system for determining the dimensions and surface relief of the spatial micrometre-sized object under examination consists of a housing 1, x and y degrees of freedom horizontal positioning device 2, the optical sensor 3, first vertical positioning device 4, microscope 5, second vertical positioning device 6, an electrode for measuring electrochemical properties 7, motion controller and data acquisition device 8, computer 9, display 10, examined spatial micrometre-sized object 11, holder 12, microcomputer 13, software LinuxCNC 14, positioner drive controllers 15, potentiostat 16, the network controller 17, USB adapter 18, memory 19, algorithm 20, CPU (central processing unit) 21, control commands 22, map of topographic properties of the examined spatial micrometre-sized object 11, sample 24, clusters of micrometre-sized objects in sample 25.

Fig. 1a and Fig. 1b show the system for determining the dimensions and surface relief of the spatial micrometre-sized object examined, where the micrometre-sized object can be biological objects, for example, tissue cells of humans, animals, or unicellular organisms. The system has a housing 1 mounted on an anti-vibration table. On the horizontal part of housing 1, a horizontal positioning device 2 of two x and y degrees of freedom is set out and connected to the horizontal positioning drives (not shown in the drawing). An optical sensor 3 is attached to the horizontal positioning device 2, above which a holder 12 is mounted, for example, a petri dish, in which sample 24 is placed, including clusters of spatial micrometre-sized objects 25 (Fig. 5), where the spatial micrometre-sized object under examination 11 is one of the selected spatial micrometre-sized object clusters 25, which may consist of one or more or many tissue cells of biological objects. The first vertical positioning device 4 is arranged on the vertical part of housing 1, connected to a separate vertical positioning device (not shown in the drawing). A microscope 5 is attached to the first vertical positioning device 4, where the vertical positioning device 4 is for adjusting the focusing distance of the light beam in the z-axis direction. On the vertical part of housing 1, a second vertical positioning device 6 is located at a selected horizontal distance from the first vertical positioning device 4 and connected to another separate vertical positioning device (not shown in the drawing). An electrode 7 for measuring electrochemical properties is attached to the second vertical positioning device 6. The second vertical positioning device 6 for the mentioned electrode 7 is used to adjust the distance to the examined spatial micrometre-sized object 11 surfaces in the z-axis direction. The mentioned positioning devices (2, 4, 6) are controlled using the motion controller and data acquisition device 8, which, communicating with the computer processor 9 via the Ethernet data bus, receives movement commands and generates the control pulse of the positioning devices (2, 4, 6) according to them and forwards the received and digitally processed measurement data to the main computer 9.

Control signals for the stepper motors of the positioning devices are formed in the motion controller 8, according to the control commands 22, which are generated in the main processor 21, considering the examined spatial micrometre-sized object 11 selected by the user and the generated scanning trajectory (Fig. 3). The entire system is mounted on a base protected from environmental vibrations, usually on an anti-vibration table. Such a system makes it possible to obtain a sharp optical image and avoid blurring the contour of the object in the image. Electrode 7 for measuring electrochemical properties is mounted on a separate vertical axis, which allows it to approach the examined spatial micrometre-sized object 11, measuring the leakage current and studying the real distance from the sensitive surface of electrode 7 to the surface of the examined spatial micrometre-sized object 11. The horizontal distance from the optical axis of microscope 5 to the vertical axis of the electrochemical properties measuring electrode 7 is fixed and calibrated using a special sample and stored in the system controller as a system parameter. The motion controller 8 is composed of two parts - the positioning devices control system (13, 14, 15) and the electrochemical data collection and transmission system 16.

The system is controlled by a computer 9, which controls the microcomputer 13 that controls the actuators of the positioning devices and processes the received image from the optical microscope 5 and controls the movement trajectory of the positioner by directing the electrochemical measurement electrode to the detected micrometre-sized 11. Computer 9 is connected to motion controller 8 via Ethernet network 17 and USB adapter 18.

Processor 21 of computer 9 is configured in such a way that the measurement data of electrode 7 for measuring electrochemical properties, according to which the height of the examined micrometre-sized object and the electrochemical activity at the measured points are determined, are merged with the optical data received via the USB data bus from the microscope 5 and the optical sensor 3, and in real-time are presented on the computer display 10 and recorded in its memory. During data fusion, a three-dimensional array of measurement points is created, which stores the coordinates of the measurement points, which are aligned with the optical image using the coordinates of the positioning devices, and the height of the micrometre-sized object is found based on the electrochemical measurement data. This array is used to store spatial micrometre-sized objects. The obtained spatial measurement result of the surface of the micrometre-sized object is matched with the optical image from the microscope, thus creating a general spatial composite image of the surface of the micrometre-sized object.

The principle of operation of the system for determining the dimensions and surface relief of the micrometre-sized object under examination, the hardware composition of which is presented in Fig. 1a and Fig. 1b, is shown in Fig. 2. The operation of the system begins with the selection of the test micrometre-sized object 11 from the sample 24. After the operator starts the measurement, first, commands are given to positioning device 2 to position sample 24 in measurement field 5 of the optical microscope. The focus distance is adjusted using positioning device 4 of the vertical positioning device, and a photograph of the sample area 24 is taken. In the next step, the image from below is captured by optical sensor 3, where we see the shadow of the real-scale sample 24 and its contour. The received data from microscope 5 and optical sensor 3 are transferred to memory 19 of computer 9 through the USB adapter 18. Further, the received data are processed in the central processor CPU according to algorithm 20, which is shown in Fig. 3. From the image of the sample captured by optical sensor 3 and the microscope 5 (Fig. 5) places where clusters of micrometre-sized objects are visible (areas of interest) 25. If clusters of micrometre-sized objects are not distinguished, then an error message is sent, and the process is stopped, if clusters are detected, then one cluster of interest 25 is selected from the clusters of micrometre-sized objects as the examined spatial micrometre-sized object 11. Using positioning device 2, the examined spatial micrometre-sized object 11 is moved so that microscope 5 is above the center of the selected spatial micrometre-sized object 11 under examination, and then it is photographed by microscope 5, and according to the data of the optical sensor 3, the contour (shadow) of the selected micrometre-sized object 11 and its position in the ROI coordinates (Region of interest - local object environment coordinates).

After analysis of the images received from microscope 5 and optical sensor 3, a set of control commands 22, for positioning devices 2,4,6, and potentiostat 16 are formed in the computer processor 9, and recorded in the memory. The control commands of the potentiostat 16 and its measurement data are transmitted through USB adapter 18. The control commands for positioning devices are transmitted through the network controller 17 to the motion control and data acquisition system 8, where the microcomputer 13, using the software LinuxCNC 14, converts them into physical control signals for the actuators of positioning devices and transmits them to the actuator controller 15, where these signals are modulated accordingly and transmitted to the actuators of the positioning devices (2, 4, 6) as supply voltage. Next, the electrochemical test mode of the examined spatial micrometre-sized object 11 is selected, specifying the requested scanning parameters, the density and quantity of measurement points, and the scanning speed, if the operator does not select the micrometre-sized object 11 or specify the scanning parameters within a certain time, an error message is sent and the process is stopped. In the next step, using positioning device 2, the investigated spatial micrometre-sized object 11 is shifted so that the electrochemical properties measurement electrode 7 is above the center of the examined spatial micrometre-sized object 11. Gradually, with the vertical positioning device 6, bringing the electrochemical properties measurement electrode 7 closer to the micrometre-sized object 11 under examination, the electrochemical activity of the surface of the micrometre-sized object 11 under investigation is measured, during which the actual height of the micrometre-sized object at the selected scanning point is determined.

According to the scanning parameters entered in the computer 9 by the operator at the previous stage and the size of the examined spatial micrometre-sized object determined by the microscope 5 and the optical sensor 3, a scanning trajectory is formed, and a scan of the examined spatial micrometre-sized object 11 is performed with the electrode 7 for measuring electrochemical properties, during which the micrometre-sized object under examination 11 is moved by the positioning device 4 according to the determined trajectory in fixed-size steps of electrochemical in relation to the properties measuring electrode 7.

The step size is calculated in the main processor based on the density and number of measurement points set by the operator. Electrochemical activity is measured at each measurement point, and the resulting measurement result is associated with the real coordinates of the positioning devices at the measured point and is recorded as one element of the three-dimensional array in the computer memory.

At the end of the scan, all positioning devices are returned to their initial positions and the automatic process is stopped.

The data fusion algorithm of sensors (5, 3), positioning devices (2, 4, 6), and electrochemical measurement system (7, 16) used in the system for determining the dimensions and relief of micrometre-sized objects are shown in Fig. 4. Data fusion performed in several stages, using data from optical microscope 5, the optical sensor 3 and electrochemical measurement system consisting of electrode 7 for measuring electrochemical properties and potentiostat 16. During scanning, optical sensor 3 is used as a feedback element, according to its data, the movement of positioning device 2 is adjusted, ensuring that electrode 7 does not go outside the scanning zone.

After the scanning, according to the measurement results stored in the memory and the coordinates set in the positioning devices control commands, the final connection of all three types of data is performed in the central processor, namely the connection of the microscope 5, the optical sensor 3 and the scanning electrode for measuring electrochemical properties (7) connected to the positioning devices (2, 4, 6) position coordinates, data. The coordinates of the positioning devices (2, 4, 6) are known according to the number of scan steps that were set. By combining the data, a map of the topographic properties of the spatial micrometre-sized object under examination 23 is formed, which provides an optical image of the spatial micrometre-sized object under examination with the real coordinates of its surface points and the results of measuring its electrochemical activity at the examined scanning points, according to which the height of the examined micrometre-sized object at the mentioned points is determined. The resulting property map is saved in the computer memory and displayed on screen 10 in real-time.

CLAIMS

1. A system for determining dimensions of a spatial examined spatial micrometre-sized object and its surface relief, by generating a composite image of the examined spatial micrometre-sized object (11), comprising:

a housing in which a microscope (5) is installed,

a holder (12) for placing the examined spatial micrometre-sized object (11),

a positioning device to move the holder (12) and the microscope (5) relative to each other along the x-axis and/or along the y-axis and/or along the z-axis to place the examined spatial micrometre-sized object (11) in a selected position relative to an optical axis of the microscope (5),

a computer with a main programmable processor (21) that communicates with the microscope (5) and is programmed to obtain a general three-dimensional image of the examined spatial micrometre-sized object, **characterized in** that

the system is provided with an optical sensor (3) and an electrochemical properties measurement electrode (7), wherein the positioning device which is installed in the housing (1) comprises:

- a horizontal positioning device (2) with degrees of freedom along the x-y axes,
- a first vertical positioning means (4) having a degree of freedom along the z-axis, and
- a second vertical positioning means (6) having a degree of freedom along the z-axis and located in the housing at a selected horizontal distance from the first vertical positioning means (4), wherein

the optical sensor (3) is attached to the horizontal positioning device (2), above which a holder (12) is attached, in which the examined spatial micrometre-sized object (11) is placed,

mentioned microscope (5) is attached to the first vertical positioning means (4), and the electrochemical properties measurement electrode (7) is attached to the second vertical positioning means (6), wherein

the horizontal positioning device (2) is mounted with the possibility to move in the directions of the x-y axes and to be placed in selected positions in relation to vertical axes of the microscope (5) and the electrochemical properties measurement electrode (7), wherein during the operation of the system, when the examined spatial micrometre-sized object (11) is in the field of view of the microscope (5) it is photographed by the microscope (5) and the real-size outline of the examined spatial micrometre-sized object (11) is captured by the optical sensor (3), wherein data from the microscope (5) and the optical sensor (3) is transmited to the main processor of the computer (9) and its memory;

and

during the operation of the system when the examined spatial micrometre-sized object (11) is in the field of the measuring electrochemical properties electrode (7) it is scaned out in the x-y plane defined by the contour of the spatial micrometre-sized object (11) according to the trajectory, number of scanned points and their density set by the computer main processor (21) and is measured electrochemical properties at each scanned point, which is associated with the real coordinates of the positioning means (2) and (6) at each scanned point and data is recorded in the memory of the computer (9),

the main processor (21) of the computer (9) is programmed in such a way that the aforementioned data recorded in its memory, received from the microscope (5), optical sensor (3), and from the electrochemical properties measurement electrode (7), the data of which is linked to the coordinates of the scanned points are merged by obtaining a total spatial image of the examined spatial micrometre-sized object with its real dimensions and its surface relief.

2. The system according to claim 1, **characterized** in that the correlation of the electrochemical activity measured by the electrochemical properties measurement electrode (7) at the scanned point with the real coordinates of the positioning means (2, 6) at each scanned point is obtained according to the control commands (22) which are generated in the main processor (21), taking into account the size and shape of the spatial micrometre-sized object (11) selected by the user, thereby creating a trajectory for scanning electrochemical properties with the electrochemical properties measurement electrode (7).

3. The system according to any one of claims 1-2, **characterized** in that the examined spatial micrometre-sized object (11) is selected from biological objects,

such as human, animal, or unicellular tissue cells.

4. The system according to claim 3, **characterized** in that the examined spatial micrometre-sized object (11) includes one, several, or many of said cells.

5. A method of determining the dimensions of a examined spatial micrometre-sized object and its surface relief by generating a composite image of the spatial micrometre-sized object (11) using a microscope (5), **characterized** in that the method comprises the following steps:

- taking an image of a top view of the examined spatial micrometre-sized object (11) with a microscope (5) and transferring the data to the memory of the computer (9),
- capturing a real-size contour of the examined spatial micrometre-sized object (11) with an optical sensor (3),
- moving the examined spatial micrometre-sized object (11) into the measuring field of an electrochemical properties measurement electrode (7),
- scanning the examined spatial micrometre-sized object (11) by electrochemical properties measurement electrode (7) in the x-y plane defined by the examined spatial micrometre-sized object (11) contour according to the trajectory and the set number of scanned points and their density set by the computer (9) main processor (21), wherein at each scanned point the electrochemical properties measurement electrode (7) measures the electrochemical activity, which determines the height of the examined spatial micrometre-sized object at the scanned point,
- linking the measured electrochemical activity at each point with the real coordinates of each point and transferring the obtained data to the main computer (9) memory,
- thanks to the program installed in the computer main processor (21) merging of data obtained from the microscope (5), the optical sensor (3), and the data from the electrochemical properties measurement electrode (7), respectively associated with the coordinates of each scanned point, by obtaining a general image of the examined spatial micrometre-sized object with its real dimensions and its surface relief.

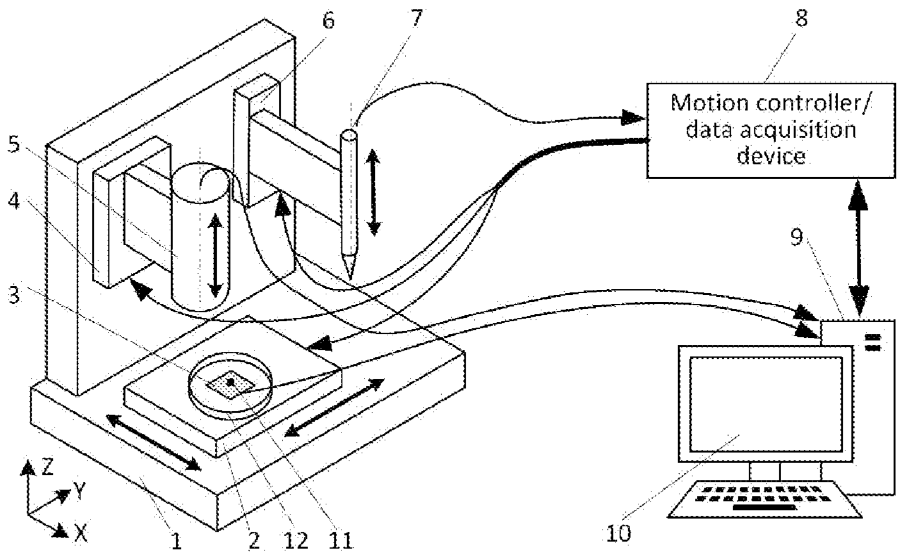
6
7
8
Motion controller/
data acquisition
device
5
4
3
9
Z
Y
X
1
2
12
11
10

Fig.1a

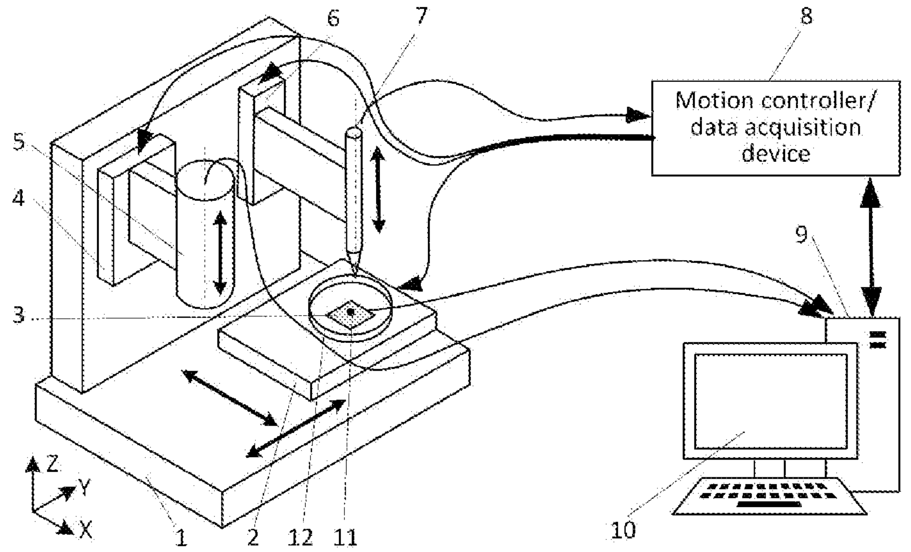
6
7
8
Motion controller/
data acquisition
device
5
4
3
9
Z
Y
X
1
2
12
11
10

Fig.1b

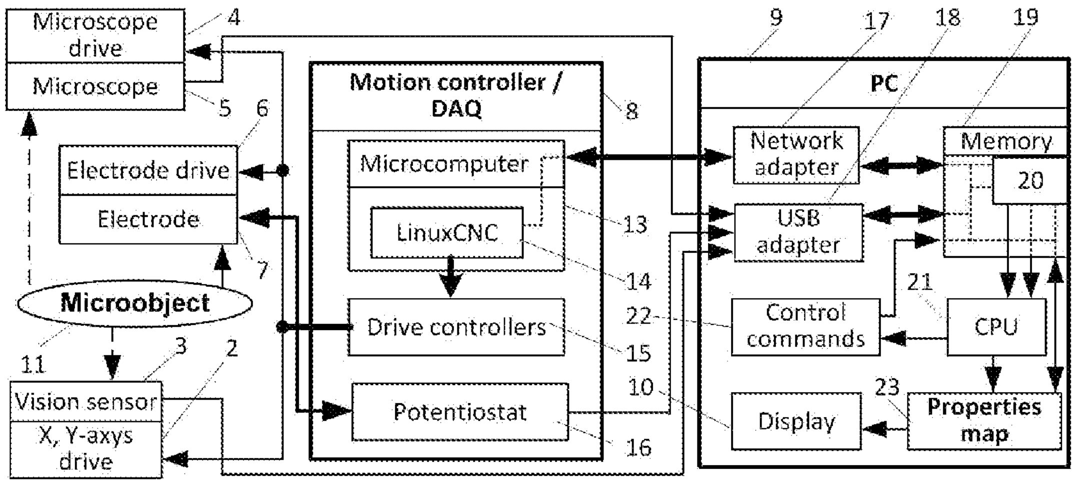
Microscope drive
Microscope
Electrode drive
Electrode
Microobject
Vision sensor
X, Y-axys drive
Motion controller / DAQ
Microcomputer
LinuxCNC
Drive controllers
Potentiostat
PC
Network adapter
USB adapter
Memory
20
Control commands
CPU
Display
Properties map
4
5
6
7
8
9
10
11
2
3
13
14
15
16
17
18
19
21
22
23

Fig.2

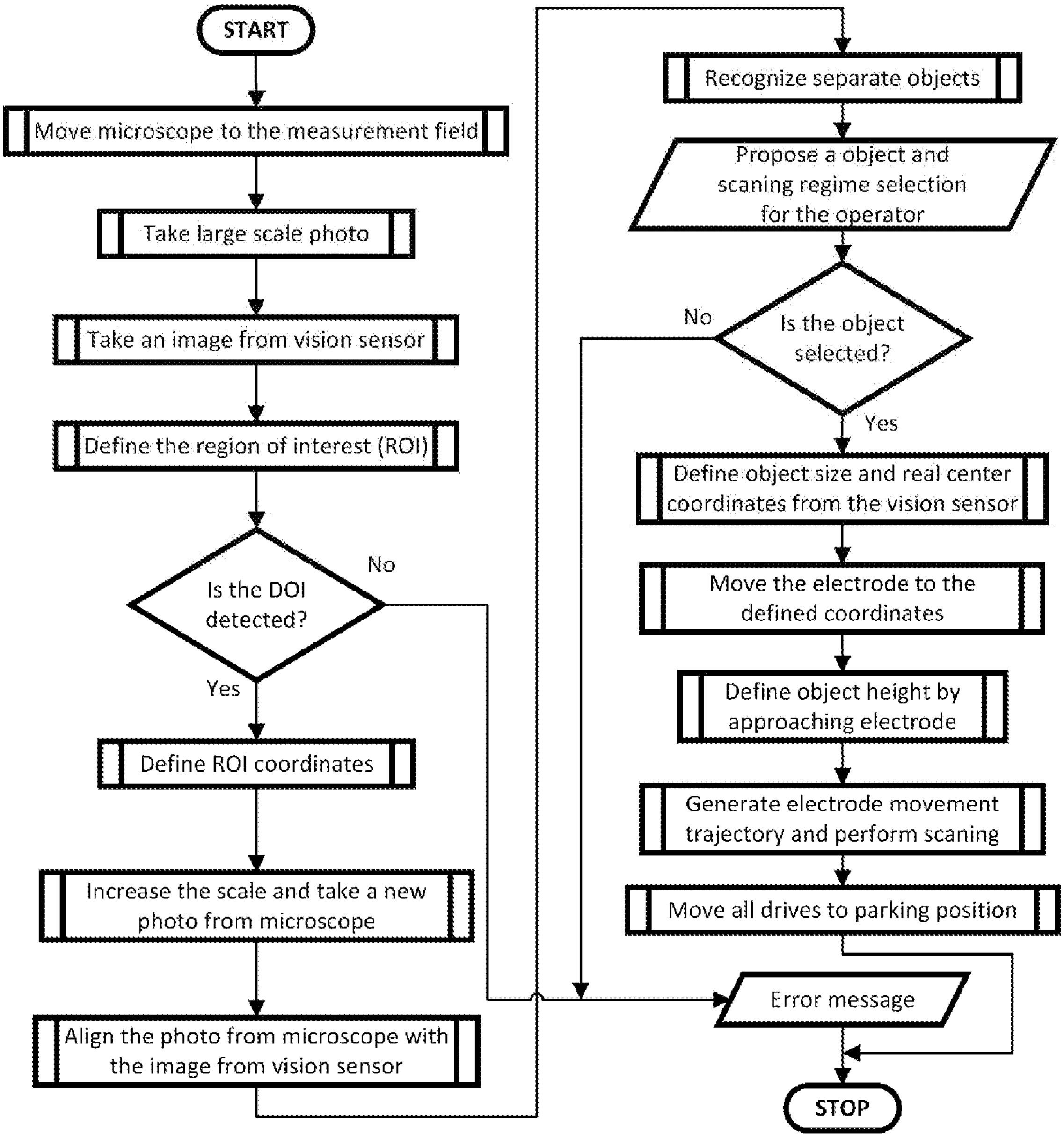
START
Move microscope to the measurement field
Take large scale photo
Take an image from vision sensor
Define the region of interest (ROI)
Is the DOI detected?
No
Yes
Define ROI coordinates
Increase the scale and take a new photo from microscope
Align the photo from microscope with the image from vision sensor
Recognize separate objects
Propose a object and scaning regime selection for the operator
Is the object selected?
No
Yes
Define object size and real center coordinates from the vision sensor
Move the electrode to the defined coordinates
Define object height by approaching electrode
Generate electrode movement trajectory and perform scaning
Move all drives to parking position
Error message
STOP

Fig.3

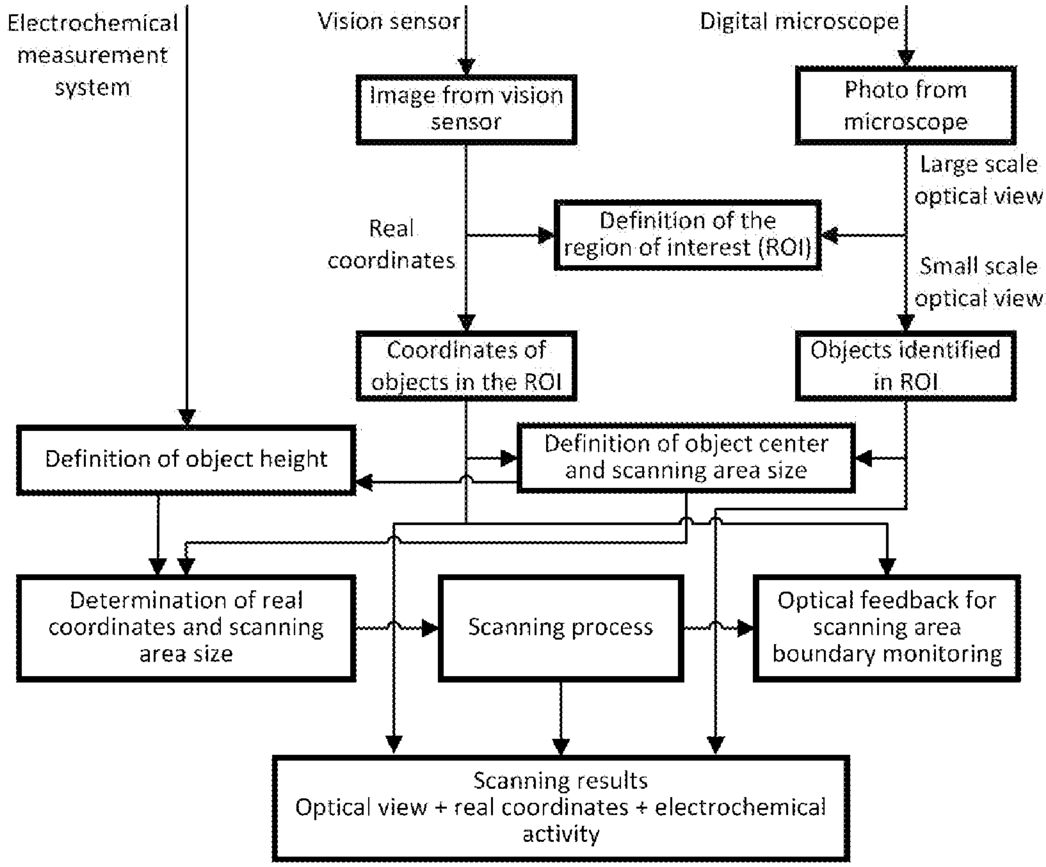
Electrochemical measurement system
Vision sensor
Digital microscope
Image from vision sensor
Photo from microscope
Large scale optical view
Real coordinates
Definition of the region of interest (ROI)
Small scale optical view
Coordinates of objects in the ROI
Objects identified in ROI
Definition of object height
Definition of object center and scanning area size
Determination of real coordinates and scanning area size
Scanning process
Optical feedback for scanning area boundary monitoring
Scanning results
Optical view + real coordinates + electrochemical activity

Fig. 4

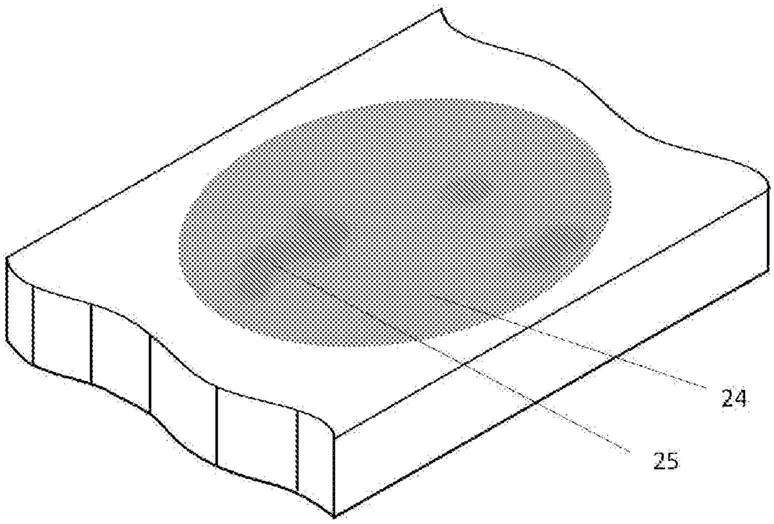

Fig.5

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2023/056358

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01B11/245 G01B11/24 G01N15/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01B G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HIRANO YU ET AL: "Construction of Time-Lapse Scanning Electrochemical Microscopy with Temperature Control and Its Application To Evaluate the Preservation Effects of Antifreeze Proteins on Living Cells", ANALYTICAL CHEMISTRY, vol. 80, no. 23, 1 December 2008 (2008-12-01), pages 9349-9354, XP93086956, US ISSN: 0003-2700, DOI: 10.1021/ac8018334 the whole document ----- -/--	1-5

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 September 2023	13/10/2023
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Kokkonen, Jukka

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 2005 201748 A (SHARP KK) 28 July 2005 (2005-07-28) abstract paragraph [0001] - paragraph [0091] figure 6 -----	1-5
A	WO 2021/196419 A1 (SUN LIANG [CN]) 7 October 2021 (2021-10-07) abstract page 1 - page 21 figures 7-16 -----	1-5

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2023/056358

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
JP 2005201748	A	28-07-2005	NONE		
WO 2021196419	A1	07-10-2021	CN 111256616	A	09-06-2020
			WO 2021196419	A1	07-10-2021