High-speed atomic force microscope, preferably for dermatological measurements

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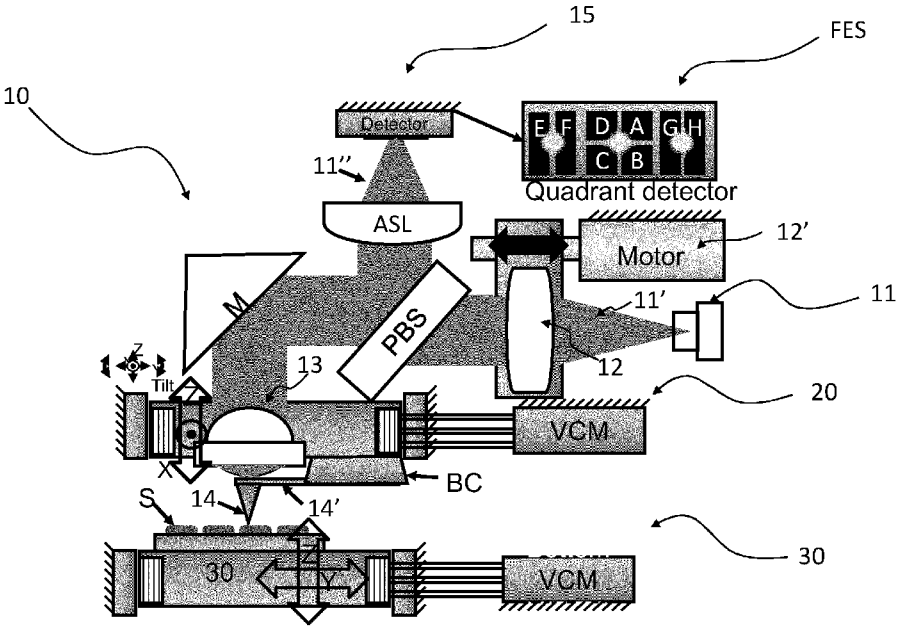

FIG. 1

(57) Abstract: The invention relates to a high-speed atomic force microscope (AFM, 1) for measurement of an associated sample (S), such as a skin sample, the AFM has a sensor unit (SU, 10) with a light source (11) and a collimator lens (12), the collimating lens is mechanically displaceable with a collimator lens actuator (12'). An AFM cantilever with an AFM tip (14) is arranged relative to photo detection beams (15) for providing a focus error signal (FES) indicative of mechanical engagement with the sample. The collimator lens (12) is arranged for being adjusted by the collimator lens actuator (12') along the optical beam path so as to compensate at least for thermal drift in the focus error signal (FES). The invention is advantageous for obtaining an improved high-speed AFM by this internal thermal drift compensation mechanism. The high-speed AFM of one embodiment of the invention may have a theoretical linear scanning speed that can reach 700,000 μm/s, which allows one imaging to be completed in around 3.4 seconds.

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HIGH-SPEED ATOMIC FORCE MICROSCOPE, PREFERABLY FOR DERMATOLOGICAL MEASUREMENTS

FIELD OF THE INVENTION

The present invention relates to a high-speed atomic force microscope (AFM), preferably for dermatological measurements, in particular an atomic force microscope capable of measuring skin morphology, such as dysfunctions in the skin barrier of a human being, in a non-invasive manner. The inventions also relates to a corresponding method and a corresponding computer program for implementing the present invention.

BACKGROUND OF THE INVENTION

The integrity of the skin barrier is essential for healthy skin, and its dysfunction can lead to Atopic dermatitis (AD), which affects more than 790 million people. A robust, pain-free, unlike biopsy, non-invasive assay, mostly macroscopic, is preferred for the AD assessment. Atomic force microscopy (AFM) is a powerful tool for the study of nanoscale materials and surfaces. The technique operates by using a sharp tip (end radius <8 nm) attached to a cantilever to probe the surface of various types of samples, including biological specimens, polymers, and inorganic materials and measure the forces between the tip and the sample to generate nanometer even atomic scale high-resolution images of the sample's surface.

Tape-stripped corneocyte can be imaged using AFMs. In recent years, AFM was employed to explore the nanoscale anatomy, which yielded nano-objects as a biomarker. AFM is a suitable tool to detect any change in corneocyte topography on the lesion and non-lesion skin in a non-invasive manner. In addition, the native-state corneocytes nanoscale texture (nanotexture) can be imaged without the need for a vacuum environment or sample preparation that are required for scanning electron microscopy. However, conventional AFMs suffer from low throughput (5-10 minutes per image), high cost, and is unsuitable for on-site skin measurement, making it inappropriate for clinical testing.

High-speed atomic force microscopy (HS-AFM) has been developed to overcome these limitations and increase the imaging speed of AFM. HS-AFM can be achieved by improving the scanning speed, reducing the cantilever damping, and increasing the cantilever resonance frequency. HS-AFM has been successfully applied to a wide range of materials and surfaces, including dynamic imaging of living cells, high-speed imaging of soft materials, and in situ imaging of reaction processes. One of the key factors in increasing the imaging speed of AFM is the development of faster scanning systems. Early AFM systems used slow piezoelectric scanning mechanisms, which limited the imaging speed. In recent years, the development of high-speed piezoelectric scanners and resonant scanners has greatly increased the imaging speed of AFM. For example, high-speed piezoelectric scanners use high-frequency piezoelectric actuators to rapidly scan the tip across the sample surface.

In addition to improvements in the scanning mechanism, reducing the cantilever damping and increasing the cantilever resonance frequency are also important for achieving HS-AFM. The cantilever damping can be reduced by using high quality factor cantilevers with low damping materials, such as silicon nitride. The cantilever resonance frequency can be increased by using smaller cantilever dimensions or by using material with higher Young's modulus, such as silicon. Another important factor in achieving HS-AFM is the development of specialized software for high-speed imaging. This software must be able to handle the large amounts of data generated by the high-speed scanning, and must be able to accurately track the position of the tip in real-time.

One of the key features of HS-AFM is its ability to perform dynamic imaging at high speeds. This allows the imaging of fast processes, such as the motion of cells, with high temporal resolution. In addition, HS-AFM can also be used for imaging soft materials, such as polymers and gels, which can be challenging for traditional AFM due to their viscoelastic behavior. There are several imaging modes that are commonly used in HS-AFM, including contact mode, intermittent contact mode, and tapping mode. In contact mode, the tip is brought into contact with the sample surface, and the deflection of the cantilever is measured as the tip is scanned across the surface.

However, conventional AFMs have limitations in terms of speed, cost, and applicability for on-site skin measurements, making them unsuitable for clinical testing.

A technical problem is the drift, in particular the thermal drift, of the parts and components of the AFM during a measurement. In previous work, cf. REVIEW OF SCIENTIFIC INSTRUMENTS 83, 013703 (2012), by E.-T. Hwu et al., an anti-drift and auto-alignment mechanism was applied to an astigmatic detection system (ADS)-based atomic force microscope (AFM) for drift compensation and cantilever alignment because conventional DVD optical heads suffer from signal drift problems. In previous setups, signal drifts of even thousands of nanometers had been measured. With the anti-drift and auto-alignment mechanism of the work from 2012, the signal drift was compensated by actuating a voice coil motor of the DVD optical head, and a nearly zero signal drift was achieved.

WO2018109803 (Olympus Corp.) relates to an atomic force microscope is provided with an XY movable stage; an XY scanner for causing the XY movable stage to move in an XY scanning motion within a plane parallel to an XY plane; a Z scanner that has a fixed end held by the XY movable stage, has a free end that holds a cantilever, and causes the cantilever to move in a Z scanning motion along a Z axis perpendicular to the XY plane; and an optical displacement sensor for optically detecting the displacement of the cantilever. The optical displacement sensor includes a converging lens held by the XY movable stage. The converging lens converges incident detection light, generates detection light having a focused spot, and irradiates the generated detection light onto the cantilever. The atomic force microscope is further provided with a Z scanning tracking system for, in accordance with the Z scanning, suppressing the movement of the position of an irradiation area along the cantilever that is caused by the Z scanning. The optical detection is based on a two-split detector with a so-called position sensitive detector (PSD), cf. Figures 1, 12 and 22, which can track position changes of the optical reflection spot from the cantilever, but it can effectively not track any thermal drift in the AFM setup.

Other research groups have attempted to perform high speed AFM without compensation of thermal results in complicated measurements setups with very long waiting time for thermal equilibrium, for example hours of waiting time. Thus, there is a need for a high-speed AFM with effective thermal drift compensation.

Hence, an improved AFM would be advantageous, and in particular a more efficient and/or reliable high-speed AFM for skin measurements would be advantageous.

OBJECT OF THE INVENTION

It is a further object of the present invention to provide an alternative to the prior art.

In particular, it may be seen as an object of the present invention to provide a high-speed AFM that solves the above mentioned problems of the prior art with thermal drift compensation and/or for performing dermatological measurements in an effective way.

SUMMARY OF THE INVENTION

Thus, the above described object and several other objects are intended to be obtained in a first aspect of the invention by providing a high-speed atomic force microscope (AFM) for measurement of an associated sample, the AFM comprising:

- a sensor unit comprising
    - a light source capable of emitting a light beam,
    - a collimator lens arranged for receiving and collimating said light beam on an optical path towards said associated sample, said collimating lens being mechanically displaceable with a corresponding collimator lens actuator,

- an objective lens receiving and focusing said light beam on an optical path towards said sample, said objective lens being mechanically displaceable with a corresponding objective lens actuator,
- an AFM cantilever with an AFM tip arranged for mechanical engagement with the associated sample, a rear side of the AFM cantilever being arranged for optically reflecting said light beam received from said objective lens in response to the said mechanical engagement, and
- photo detection beams for detecting said reflected light from the AFM cantilever and providing a focus error signal (FES) indicative of said mechanical engagement,

- a top scanning actuator, preferably a voice coil motor, for at least one dimensional displacement of the sensor unit (SU) in a scanning direction (X) and displacement in an approaching direction (Z) towards the associated sample, and

- a sample stage for mounting said associated sample,

wherein the top scanning actuator and the sample stage are arranged for at least two-dimensional (X-Y) scanning of the surface of the sample using the sensor unit, and wherein the collimator lens is arranged for being adjusted by said collimator lens actuator along the optical beam path so as to compensate at least for thermal drift in the focus error signal (FES).

The invention is particularly, but not exclusively, advantageous for obtaining a more efficient and reliable high-speed AFM by providing a novel and advantageous internal thermal drift compensation mechanism. The high-speed AFM of one embodiment of the invention has a full working range of $1.4 \times 1.4 \times 3.8$ mm^3 and a theoretical linear scanning speed that can reach 700,000 µm/s, which allows one imaging to be completed in around 3.4 seconds.

For dermatological measurements, the atomic force microscope of the present invention is capable of measuring skin morphology by imaging nano-textures of e.g. skin corneocytes with a constant height scanning mode in just seconds. The high-speed AFM can therefore potentially be an Atopic Dermatitis Quality of Care

(ADQoC) initiative recommended system for skin disorders, such as AD, preventive risk estimation, and treatment efficacy assessment.

Definitions:

In the context of the present invention, it is to be understood that the term atomic force microscopy (AFM) is be interpreted broadly as comprising various kind of scanning probe microscopy (SPM) with a resolution in order of fraction of nanometers. AFM was originally developed by Binnig, Quate and Gerber in 1986, and the skilled person is assumed to be familiar with the basic concept and principle of the AFM, the skilled person being referred to for example N. C. Santos and F. A. Carvalho, "Atomic Force Microscopy - Methods and Protocols." pp. 1–369, 2019, which is hereby included by reference in its entirety. Additionally, the 3 references:

- Ando, T. and Uchihashi, T. (2015). High-speed atomic force microscopy coming of age. Nanotechnology, 26(43), 434001. DOI: 10.1088/0957-4484/26/43/434001, and
- Eghiaian, F., Rico, F., Colom, A., Casuso, I., & Scheuring, S. (2014). High-speed atomic force microscopy: Imaging and force spectroscopy. FEBS letters, 588(19), 3631-3638. (https://doi.org/10.1016/j.febslet.2014.06.028), and
- Ando, T., Uchihashi, T., Kodera, N., Yamamoto, D., Taniguchi, M., Miyagi, A., & Yamashita, H. (2007). High-speed atomic force microscopy for observing dynamic biomolecular processes. Journal of Molecular Recognition, 20(6), 448-458. (https://doi.org/10.1002/jmr.843),

are hereby included by reference in their entirety, and the skilled person can find more information the principle of AFM, and in particular the use of high-speed AFM for imaging of biological materials and surfaces.

In the context of the present invention, it is to be understood that the term displacing is considered to include the meaning of relatively displacing, i.e. a first object may be displaced with respect to a second object meaning that the first object may be moving and the second object can be stationary, or vice versa, or that both the first and second object may be moving in relation to each other.

In the context of the present invention, it is to be understood that thermal drift is caused by small thermal changes and/or fluctuations between parts of the AFM system and the surroundings. Thus, thermal drift also includes characteristics, parameters, and/or operation of electronics and/or optical components applied in the AFM system changing because of thermal differences and fluctuations. Thus, for example aluminium has a linear thermal expansion coefficient of 24x10-6 K/m, and thus one centimetre aluminium will expand 240 nm when temperature rises one degree K. But some typical plastic materials has 10 times larger linear thermal expansion coefficient. Some internal sources of heating in a typical AFM setup may include; laser diodes, sensors, photo sensors, etc. Some external sources of heating in a typical AFM setup may include; environmental/surrounding temperatures, air conditions.

The present invention may in particular be applied for skin measurements, thus, the AFM may be applied for measuring cornelcyte surface nanotexture patterns. Using the present invention to identify nanoscale features and correlate to skin diseases is a desired option. The first proven nanoscale feature may be circular, or other shapes, of nano-texture which is correlated to the following non-limited list of skin barrier dysfunction related diseases:

- Atopic dermatitis (AD) as mentioned above.

- Eczema (atopic dermatitis): It is a chronic inflammatory skin condition that causes red, itchy, and inflamed skin.

- Psoriasis: It is an autoimmune disorder that causes the skin cells to grow rapidly, leading to thick, scaly patches of skin.

- Acne: It is a common skin condition that causes pimples, blackheads, and whiteheads.

- Rosacea: It is a chronic inflammatory skin condition that causes redness, flushing, and small, red, pus-filled bumps on the face.

- Contact dermatitis: It is a skin condition that occurs when the skin comes into contact with an irritant or allergen, causing redness, itching, and inflammation.

- Ichthyosis: It is a genetic disorder that causes dry, scaly skin.

- Xerosis: It is a medical term for dry skin, which can cause itching, cracking, and flaking.

- Seborrheic dermatitis: It is a common skin condition that causes red, scaly patches and dandruff.

- Cutaneous lymphoma: It is a rare type of skin cancer that affects the immune cells in the skin.

It is contemplated that the present invention may enable large scale data collection of skin samples with various medical conditions/diseases i.e. one or more of the above mentioned medical condition(s), and based on such data collections with characteristic texture and morphology of these medical conditions/diseases may be applied in texture databases with these medical conditions/diseases. A large amount of data in a nanotexture database can help correlating skin medical conditions/diseases to different size/shape/feature nano-textures, cf. for example C. Riethmüller, "Assessing the skin barrier via corneocyte morphometry," Exp. Dermatol., vol. 27, no. 8, pp. 923–930, 2018, doi: 10.1111/exd.13741. Additionally, it is contemplated that artificial intelligence or machine learning can be applied for characterizing texture databases and correlated with relevant medical conditions/diseases.

Though the present invention has been implemented for AFM contact mode imaging, there are various embodiments and details of contact mode AFM that the skilled person will readily understand can be combined with the present invention once the general principle and teaching of the present invention is fully understood. Thus, some non-limiting contact AFM mode variants and embodiments may include:

- High Resolution: Contact mode provides high resolution images of the sample surface with high vertical and lateral resolution.

- High Force Sensitivity: The AFM tip exerts a force on the sample surface, and the resulting deflection of the cantilever is used to determine the topography of the surface. The force sensitivity of the AFM tip is typically in the range of picoNewtons, which allows the measurement of very small forces.

- High Wear: Contact mode AFM can cause wear and damage to both the AFM probe and the sample surface due to the direct contact between them.

- Slow Scanning: Contact mode AFM requires a slow scanning speed due to the high forces exerted on the sample surface. This slow scanning speed can limit the imaging speed and can also increase the risk of sample damage.

- Limited for soft materials: Contact mode AFM may not be suitable for imaging soft materials or delicate samples, as the high forces exerted by the AFM probe can deform or damage the sample.

These are some of the key characteristics of contact mode AFM. While contact mode AFM may provide high resolution images, it may not be suitable for all samples or applications. Other modes of AFM, such as non-contact mode or tapping mode, can be used to minimize the damage to the sample and improve the imaging speed.

Moreover, it is contemplated that other embodiments or modes than contact AFM that the skilled person will readily understand can be combined with the present invention once the general principle and teaching of the present invention is fully understood. Other modes than contact mode for operating an AFM according to the present invention may include these non-limiting modes:

- Non-contact mode: In this mode, the AFM tip is brought close to the surface of the sample, but it does not touch the surface. The tip is then

oscillated at a resonant frequency, and the amplitude of the oscillation is measured. The amplitude of the oscillation is related to the distance between the tip and the surface, which is used to create a topographic map of the sample surface.

- Tapping mode: In this mode, the AFM tip is oscillated at a frequency slightly lower than its resonant frequency, so that it gently taps the sample surface. The amplitude of the tapping motion is kept constant by adjusting the feedback signal. This mode is less damaging to the sample than contact mode and is often used to image soft or delicate samples.

- Dynamic force mode: In this mode, the AFM tip is brought close to the surface and is oscillated at a frequency slightly above its resonant frequency. The amplitude of the oscillation is measured and used to control the tip-sample distance. This mode is useful for studying the mechanical properties of materials.

- Phase imaging mode: In this mode, the phase difference between the oscillation of the tip and the sample surface is measured. This mode is useful for imaging samples with different mechanical and electrical properties.

- Electrostatic force microscopy (EFM): In this mode, the AFM tip is used to measure the electric field above the surface of a sample. This mode is useful for studying the electrical properties of materials.

In advantageous embodiments, the compensating for – at least - the thermal drift in the focus error signal (FES) may be performed in a control loop mechanism based on the focus error signal and on displacement, preferably linear displacement, of said collimating lens by said collimator lens actuator. As it will explained below in the detailed description, this will provide high-speed imaging, e.g. seconds, in a relatively high quality, such as a quality effectively allowing for clinical use of the HS-AFM according to the invention for dermatological measurements of patients. In preferred embodiments, a control loop mechanism may have an optimum sensing point of a corresponding control loop curve,

preferably an optimum sensing region, as it will explained below in the detailed description in connection with Figure 2.

Advantageously, the photo detection means comprises a quadrant detector arranged for sensing a focus error signal (FES) with for example four photodetectors arranged in a two-by-two pattern with each photodetector being of the substantially same size, e.g. of a quandrangle-shape or a square-like shape, or the four detectors collectively forming a substantially circular detector, each photodetector being of the substantially same size and forming a circle section of approximately 90 degrees of the circular detector . Additionally, the quadrant detector may be optically arranged relative to a corresponding astigmatic lens (ASL) for sensing the reflected light from the cantilever like in a typically setup for detecting a focus error signal (FES) known from optical recording technology.

In preferred embodiments, the AFM may have the sample stage mechanically connected to a bottom scanning actuator, more preferably a voice coil motor, said bottom scanning actuator being further arranged for at least one dimensional displacement (Y), optionally also two-dimensional scanning (X-Y) for scanning of the surface of the associated sample relative to the sensor unit (SU). Preferably, the bottom scanning may further be arranged for displacement in the approaching direction (Z) between the associated sample and the sensor unit (SU), such as for coarse displacement in the Z-direction.

Advantageously, the AFM tip may be arranged for being scanned across the associated sample together with the objective lens in sensor unit (SU) for a compact design of the AFM.

Typically, the AFM according to the present invention may be capable of being operated in a contact mode, a non-contact mode, a tapping mode, a dynamic force mode, a phase imaging mode and/or as an electrostatic force microscopy (EFM) as the skilled person in AFM will readily understand once the general principle and teaching of the present invention is understood.

In advantageous embodiments, the working range of the top scanning actuator, and optionally the bottom scanning actuator, may enable scanning of at least 0.5

mm, preferably 0.75 mm, most preferably at least 1.0 mm, in one or two directions in the plane of performing the AFM scanning. These working ranges may advantageously enable dermatological measurements of skin samples from patients in a clinical context and environment. Additionally, these working ranges beneficially enable other surface measurements of nano-structures in the height (Z) direction, but on a macro-level in the scanning directions (X-Y).

Advantageously, the AFM according to the present invention facilitates that the linear scanning speed may at least 50.000 micrometers/second, preferably at least 100.000 micrometers/second, most preferably at least 200.000 micrometers/second. In some embodiments, even very high speeds of even 700.000 micrometer/second or higher may be reached. One of the important factors is the resonance of the actuator, which must be controlled accordingly. Thus, 700.000 micrometer/second can translate to at least 42 frames per second if scanning is done in 128x128 pixels.

Beneficially, the AFM according to the present invention may be arranged for dermatological measurements of an associated skin sample. The above non-limiting list of various diseases, and medical conditions, may thus be measured and investigated using the HS-AFM of the present invention.

In some particular embodiments, the AFM may be arranged for scanning of a sample surface under liquid conditions with at least a part of the associated sample being immersed in a liquid, where the AFM may be capable of adjusting a focal point of the optical path towards the sample by displacing said collimator lens and/or by displacing said objective lens to at least partly compensate for said liquid conditions. Thus, the collimator lens may compensate liquid evaporating and/or thermal changes or other liquid changes, e.g. chemical or bio-chemical reactions in the liquid, and/or chemical or bio-chemical reactions between the sample and the liquid.

In a second aspect, the invention relates to a method for operating a HS-AFM and performing measurements of a sample (S), preferably dermatological measurement of a skin sample, the method comprising

- providing a sensor unit (SU) comprising
  - a light source capable of emitting a light beam,
  - a collimator lens arranged for receiving and collimating said light beam on an optical path towards said associated sample, said collimating lens being mechanically displaceable with a corresponding collimator lens actuator,
  - an objective lens receiving and focusing said light beam on an optical path towards said sample, said objective lens being mechanically displaceable with a corresponding objective lens actuator,
  - an AFM cantilever with an AFM tip arranged for mechanical engagement with the associated sample, a rear side of the AFM cantilever being arranged for optically reflecting said light beam received from said objective lens in response to the said mechanical engagement, and
  - photo detection beams for detecting said reflected light from the AFM cantilever and providing a focus error signal (FES) indicative of said mechanical engagement,

- providing a top scanning actuator, preferably a voice coil motor, for at least one dimensional displacement of the sensor unit (SU) in a scanning direction (X) and displacement in an approaching direction (Z) towards the associated sample, and

- providing a sample stage for mounting said associated sample,

wherein the top scanning actuator and the sample stage are mutually arranged for at least two-dimensional (X-Y) scanning of the surface of the sample (S) using the sensor unit (SU), and wherein the collimator lens is adjusted by said collimator lens actuator along the optical beam path so as to compensate at least for thermal drift in the focus error signal (FES).

This aspect of the invention is particularly, but not exclusively, advantageous in that the present invention may provide a method for operating and measuring with a high-speed AFM by providing an advantageous internal thermal drift compensation mechanism.

In a third aspect, the invention relates to a computer program product being adapted to enable a computer system comprising at least one computer having data storage means in connection therewith to control an AFM according to the first aspect of the invention, such as a computer program product comprising instructions which, when the program is executed by a computer, cause the computer to carry out the method of second aspect of the invention.

This aspect of the invention is particularly, but not exclusively, advantageous in that the present invention may be accomplished by a computer program product enabling a computer system to carry out the operations of the apparatus/system of the first aspect of the invention when down- or uploaded into the computer system. Such a computer program product may be provided on any kind of computer readable medium, or through a network.

The individual aspects of the present invention may each be combined with any of the other aspects. These and other aspects of the invention will be apparent from the following description with reference to the described embodiments.

BRIEF DESCRIPTION OF THE FIGURES

The AFM according to the invention will now be described in more detail with regard to the accompanying figures. The figures show one way of implementing the present invention and is not to be construed as being limiting to other possible embodiments falling within the scope of the attached claim set.

Figure 1 shows a schematic drawing of an AFM embodiment according to the present invention,

Figure 2 shows a graph with the FES of an AFM as a function of the collimator adjustment,

Figure 3 shows representative some AFM images with and without the present invention,

Figure 4 is photo of an AFM according to the present invention with top sensor unit and the lower sample stage,

Figure 5 shows a schematic drawing of another AFM embodiment according to the present invention,

Figure 6 shows a schematic drawing of yet another AFM embodiment according to the present invention, and

Figure 7 is a schematic system-chart representing an out-line of/in detail the operations of the method according to the invention.

DETAILED DESCRIPTION OF AN EMBODIMENT

Figure 1 shows a schematic drawing of an AFM embodiment according to the present invention; i.e. a high-speed atomic force microscope (AFM) 1 for measurement of an associated sample S.

A sensor unit (SU) 10 thus comprises a light source 11 capable of emitting a light beam 11', such as a laser beam, and a collimator lens 12 is arranged for receiving and collimating said light beam on an optical path towards the associated sample, the collimating lens being mechanically displaceable with a corresponding collimator lens actuator 12' (schematically indicated with a bold arrow parallel to the optical path), and an objective lens 13 for receiving and focusing said light beam on an optical path towards said sample S, said objective lens 13 being also mechanically displaceable with a corresponding objective lens actuator as schematically shown. On the optical path toward the sample S, the light 11' to the sample (and reflected light 11'') can be redirected or reflected by one or more mirror(s) M as schematically shown in Figure 1.

Further an AFM cantilever 14' with an AFM tip 14 is arranged for mechanical engagement with the associated sample S, and a rear side of the AFM cantilever being arranged for optically reflecting said light beam 11'' received from said objective lens 13 in response to the said mechanical engagement. Corresponding photo detection beams 15 is arranged for detecting this reflected light 11'' via the

polarizing beam splitter PBS and the astigmatic lens ASL from the AFM cantilever 14' and providing a focus error signal FES indicative of said mechanical engagement i.e. the displacement of the AFM tip 14 as a function of the surface structure of the sample S.

Additionally, a top scanning actuator 20, preferably a voice coil motor VCM, is provided for at least one dimensional displacement, optionally two dimensional displacement, of the sensor unit (SU) 10 in a scanning direction (X) and displacement in an approaching direction (Z) towards the sample S.

Furthermore, a sample stage 30 is arranged for mounting said associated sample S. If the sample S is a skin sample, it may be positioned on top of a piece of tape between the sample stage and the sample itself, as it is also schematically indicated in the figure. The sample stage 30 is mechanically connected to a bottom scanning actuator, more preferably a voice coil motor VCM, said bottom scanning actuator being further arranged for at least one dimensional displacement (Y) for scanning of the surface of the associated sample relative to the sensor unit (SU) 10, preferably also displaceable in the approaching direction between the sample S and the sensor unit 10 i.e. in the Z-direction as also schematically indicated in the figure.

The top scanning actuator 20 and the sample stage 30 are then in combination arranged functionally and/or structurally for two-dimensional (X-Y) scanning of the surface of the sample S using the sensor unit (SU) 10 , and especially the collimator lens 12 is arranged for being adjusted by said collimator lens actuator 12 along the optical beam path so as to compensate at least for thermal drift in the focus error signal (FES) detected by the photo detection means 15.

Preferably, the photodetection means 15 comprises a two-dimensional detector arranged for being providing a focus error signal (FES), preferably from a beam splitting means ASL dividing said light beam into three different sub-beams as schematically indicated in the upper right corner of Figure 1. Additionally, the AFM according to the present invention may comprise a controller (not shown for clarity) operably connected to the sensor unit SU 10, the top scanning actuator 20, and the sample stage with the bottom scanning actuator 30 for controlling the

AFM measurements, and enabling creating an AFM image of the sample S based on the mechanical engagement detected in the focus error signal (FES).

In some embodiments, the sensor unit may use one or more parts from CD, DVD, and Blu-ray data storage optical pickup units (OPU), especially Blu-ray OPUs because the collimator lens is already displaceable in Blu-ray OPUs. The CD, DVD, and Blu-ray data storage optical pickup unit (OPU) are used to read and write data on optical discs as the skilled person will know. The OPU combines a laser diode, photodetector, and lens system to focus the laser beam onto the disc and read the data. The CD, DVD, or Blu-ray OPU can be used as the core optics in an AFM setup, cf. E. E. te Hwu and A. Boisen, "Hacking CD/DVD/Blu-ray for Biosensing," ACS Sens, vol. 3, no. 7, pp. 1222–1232, 2018, doi: 10.1021/acssensors.8b00340, where the skilled reader can find more information about this issue, and which is hereby incorporated in its entirety. The OPU can be used to measure the position of the tip and collect data from the interaction between the tip 14 and the sample S. By using the OPU as the core optics in an AFM, it is possible to improve the speed, accuracy, and/or resolution of the AFM imaging. In particular, the present invention has in one embodiment been implemented using a Blu-ray optical pickup unit (OPU) for high-speed dermatological AFM (HD-AFM) specialized for skin nanotexture imaging.

Figure 1 shows the HD-AFM fixes an AFM probe on a top voice coil motor (VCM) moving part that carries the objective lens 13 for Z and X axes movement. Thus, the tip 14 and the cantilever 14' together with a body chip BC forms an AFM probe. The OPU, i.e. more generally the SU 10 10, focuses a laser 11 (power: 0.42 mW, λ: 405 nm) on a cantilever 14' of the AFM probe (Mikromasch CSC38) through the objective lens. The top VCM 20 carries the AFM probe 14 and 14', approaches (Z-axis) a tip 14 to a sample S, and scans the probe in the X-axis direction. As schematically indicated to the left, the top VCM can also perform a tilt movement of the AFM probe. A bottom VCM moving part brings a sample for Z'-axis adjustment and Y-axis scanning. When the tip 14 is scanning on the sample surface with a constant height scanning AFM mode, the focused laser senses a nanoscale bending movement of the cantilever 14' through a focus error signal (FES) calculated from a quadrant detector in the photodetection means 15, as it well-known for OPU for optical recording/reading. It is contemplated that

one, or more, FES related signal can be applied in the context of the present invention. However, the OPU laser diode and environmental temperature variation may cause thermal drifts between one or more optical components that cause a random drifting of the FES. The newly developed HD-AFM uses an actuator or a motor inside the OPU to shift the collimator lens 12 and thereby fine-tune the focusing for the drift compensation, preferably without substantially changing the distance between the AFM probe and the objective lens.

Figure 2A shows a graph with the FES of an AFM as a function of this collimator adjustment. Thus, the motor 12' can precisely adjust the collimator lens 12 position and maintain the FES at the most sensitive point. When the collimator lens position is shifted by the collimator adjustment motor steps (horizontal axis), the focus error signal (FES) (vertical axis) signal moves accordingly. Thus, FES drift compensation can be done by driving the motor and maintaining the FES working at the most sensitive point (dot) inside the sensing region (area shown by the arrow with substantially linear slope).

Figure 2B (a) shows another graph of the FES signal like in Figure 2A, together with the corresponding AFM image obtained at the different adjustment steps of the collimator motor. Thus, during the scanning of a 1-D grating sample, where the micro-stepping motor embedded within the Optical Pickup Unit (OPU) was adjusted to move in increments of five steps. The current configuration allows the motor to make adjustments across 400 steps, encompassing the entire range of the S-curve of the FES signal. This adjustment alters the position of the collimator lens, which in turn modifies the focusing condition of the cantilever. The change in imaging quality can be observed during the motor's adjustment phase as depicted in Figure 2B (b). The incremental movement of five steps (from point 1 to 12) results in a shift of the collimator, thus altering the cantilever's focusing condition. The imaging contrast reaches its optimum when the Focus Error Signal (SFE) is aligned with the most sensitive point (at position 7). Since most of the points are inside the sensing region so one can still see the features of the sample. It is important to note that the variations in pitch distance are attributed to the VCM scanning mechanism and are not a consequence of motor adjustments.

The motor adjustment precision can be improved by using 1, 2, 4, 8, 16, 32, 64, 128 and 256 micro-stepping setting to achieve a maximum resolution of 102,400 (400x256) steps for a precise adjustment to compensate the thermal drift.

Figure 3 shows representative AFM images with and without the present invention i.e. HS-AFM thermal drift compensation test and skin nanotexture measurement results. The measurement time of one image (area: 17 × 17 μm2, 512 x 512 pixels) is 3.4 seconds. The Figures shows: (a) Without the collimator adjustment (b) With the collimator adjustment, and (c) Corneocyte morphology of a healthy skin corneocyte sample.

Thus, Figure 3 shows the HD-AFM measurement result of DVD data tracks and skin corneocyte samples. Due to a buffer circuit limitation of the controller, the scanning speed was limited to 150 lines/s (tip-sample speed: 5,100 μm/s). Without the collimator adjustment, the FES drifted outside the sensing region and the cantilever bending could not be measured, as shown in Figure 3(a). After the collimator adjustment, the FES was maintained inside the sensing region, and a topography image of the DVD data tracts was obtained (Figure 3(b)). The top VCM oscillated when starting sample scanning, which caused instability of the top 36 lines. Figure 3(c) shows the morphology of a healthy skin corneocyte sample. The instability was presented, but the image was stabilized after the 28th line. The nanoscale texture on the corneocyte can clearly be seen when measured at the sensitive point of the FES as shown in Figure 2.

Figure 4 is photo of an AFM according to the present invention with a top sensor unit and the lower sample stage. Figure 4 shows a photo of the HD-AFM containing two identical Blu-ray OPUs. The AFM coarse position adjustment and nanoscale resolution scanning are carried out by the X, Y, and Z, Z' axes which have a working distance of 1,400 μm and 1,900 μm, respectively. A full 1.4 × 1.4 × 3.8 mm^3 working range eliminates the need for external tip-sample approaching and sample coarse adjustment mechanisms while increasing the throughput while measuring different spots on the skin corneocyte sample. Four 16-bit digital-to-analog converter channels drive the actuation axes with a resolution of 21 nm (X, Y) and 29 nm (Z, Z'). Both top and bottom VCMs have 1st and 2nd resonant frequencies of 48 Hz and >20 kHz, respectively. When the HD-AFM scanning at 20

kHz, the full working range of the VCMs has a -38 dB reduction that can still achieve a sufficient AFM scanning range of 17.5 µm. The theoretical linear scanning speed can reach 700,000 µm/s, which is a significantly improvement of three orders of magnitude faster than conventional high-speed AFMs.

Thus, the present invention is an HD-AFM that has an internal thermal drift compensation mechanism. The tip scanning HD-AFM can image nanotextures on skin corneocytes with a constant height scanning mode in 3.4 seconds. Further improvement is needed for better stability of imaging. The HD-AFM can potentially be a global Atopic Dermatitis Quality of Care (ADQoC) initiative recommended system for skin disorders, such as AD, preventive risk estimation, and treatment efficacy assessment.

Figure 5 shows a schematic drawing of another AFM embodiment according to the present invention similar to the embodiment shown in Figure 1. Thus, the AFM according to this embodiment is arranged for scanning of a sample surface under liquid conditions with at least a part of the associated sample being immersed in a liquid L, as schematically indicated in Figure 5. The liquid may be water or other liquids, especially liquids conserving/maintaining certain biological relevant conditions of a sample S of biological interest while the sample is being scanned. The AFM is then capable of adjusting a focal point of the optical path towards the sample S by displacing said collimator lens 12 and/or by displacing said objective lens 13 to at least partly compensate for these liquid conditions. Thus, the collimator lens can compensate liquid evaporating and/or thermal changes, or other liquid changes, e.g. reactions taking place in the sample S and/or the liquid L above the sample.

Figure 6 shows a schematic drawing of yet another AFM embodiment according to the present invention. The embodiment is similar to the AFM schematically shown in Figures 1 and 5, but in this embodiment an additional light beam, e.g. a red laser 9, is applied for finding an approximate position of AFM tip 14 i.e. sample ranging. The Figure 6(a) part shows an internal dual Blu-ray optics hardware design with a voice coil motor (VCM) performing X & Z axes movement. Figure 6(b) shows an exploded view where the top VCM carries objective lens and pre-

aligned AFM tip for coarse Z-axis approach and X-axis coarse adjustment and high-speed scanning, and the bottom VCM carries a skin tape for Y-axis coarse adjustment and high-speed scanning. It is expected that such embodiments, where the optics and VCM are very compact, enables that the whole AFM system with its optomechanical parts can be handheld size, particularly well suited in clinical applications for imaging of skin. This handheld miniature HS-AFM can for example be used for skin corneocyte nanotexture imaging. The digitized corneocyte data can be further analysed for skin disease severity assessment.

Figure 7 is a schematic system-chart representing an out-line of/in detail the operations of the method according to the invention. Thus, the invention relates to a method for operating a HS-AFM and performing measurements of a sample S, preferably dermatological measurement of a skin sample, cf. Figures 1, 5, and 6, the method comprising

S1 providing a sensor unit (SU) 10 comprising

- a light source 11 capable of emitting a light beam,
- a collimator lens 12 arranged for receiving and collimating said light beam on an optical path towards said associated sample, cf. Figure 1, said collimating lens being mechanically displaceable with a corresponding collimator lens actuator,
- an objective lens 13 receiving and focusing said light beam on an optical path towards said sample, said objective lens being mechanically displaceable with a corresponding objective lens actuator,
- an AFM cantilever with an AFM tip 14 arranged for mechanical engagement with the associated sample, a rear side of the AFM cantilever being arranged for optically reflecting said light beam received from said objective lens in response to the said mechanical engagement, and
- photo detection beams 15 for detecting said reflected light from the AFM cantilever and providing a focus error signal (FES) indicative of said mechanical engagement,

S2 providing a top scanning actuator 20, preferably a voice coil motor, for at least one dimensional displacement of the sensor unit (SU) in a scanning direction (X)

and displacement in an approaching direction (Z) towards the associated sample, and

S3 providing a sample stage (30) for mounting said associated sample,

S4 wherein the top scanning actuator and the sample stage are mutually arranged for at least two-dimensional (X-Y) scanning of the surface of the sample (S) using the sensor unit (SU) 10, cf. Figures 1, 5 and 6, and

S5 wherein the collimator lens 12 is adjusted by said collimator lens actuator along the optical beam path so as to compensate at least for thermal drift in the focus error signal (FES), cf. Figure 2.

In short, the invention relates to a high-speed atomic force microscope (AFM) 1, cf. Figure 1, for measurement of an associated sample (S), such as a skin sample, the AFM has a sensor unit (SU) 10 with a light source 11 and a collimator lens 12, the collimating lens is mechanically displaceable with a collimator lens actuator 12'. An AFM cantilever with an AFM tip 14 is arranged relative to photo detection beams 15 for providing a focus error signal (FES) indicative of mechanical engagement with the sample S. The collimator lens 12 is arranged for being adjusted by the collimator lens actuator 12' along the optical beam path so as to compensate at least for thermal drift in the focus error signal (FES). The invention is advantageous for obtaining an improved high-speed AFM by this internal thermal drift compensation mechanism. The high-speed AFM of one embodiment of the invention may have a theoretical linear scanning speed that can reach 700,000 µm/s, which allows one imaging to be completed in around 3.4 seconds.

The invention can be implemented by means of hardware, software, firmware or any combination of these. The invention or some of the features thereof can also be implemented as software running on one or more data processors and/or digital signal processors.

The individual elements of an embodiment of the invention may be physically, functionally and logically implemented in any suitable way such as in a single unit, in a plurality of units or as part of separate functional units. The invention may be

implemented in a single unit, or be both physically and functionally distributed between different units and processors.

Although the present invention has been described in connection with the specified embodiments, it should not be construed as being in any way limited to the presented examples. The scope of the present invention is to be interpreted in the light of the accompanying claim set. In the context of the claims, the terms "comprising" or "comprises" do not exclude other possible elements or steps. Also, the mentioning of references such as "a" or "an" etc. should not be construed as excluding a plurality. The use of reference signs in the claims with respect to elements indicated in the figures shall also not be construed as limiting the scope of the invention. Furthermore, individual features mentioned in different claims, may possibly be advantageously combined, and the mentioning of these features in different claims does not exclude that a combination of features is not possible and advantageous.

Some other embodiments:

E1. A high-speed atomic force microscope (AFM, 1) for measurement of an associated sample (S), the AFM comprising:

- a sensor unit (SU, 10) comprising
  - a light source (11) capable of emitting a light beam,
  - a collimator lens (12) arranged for receiving and collimating said light beam on an optical path towards said associated sample, said collimating lens being mechanically displaceable with a corresponding collimator lens actuator,
  - an objective lens (13) receiving and focusing said light beam on an optical path towards said sample, said objective lens being mechanically displaceable with a corresponding objective lens actuator,
  - an AFM cantilever with an AFM tip (14) arranged for mechanical engagement with the associated sample, a rear side of the AFM cantilever being arranged for optically reflecting said light beam received from said objective lens in response to the said mechanical engagement, and
  - photo detection beams (15) for detecting said reflected light from the AFM cantilever and providing a focus error signal (FES) indicative of said mechanical engagement,

- a top scanning actuator (20), preferably a voice coil motor, for at least one dimensional displacement of the sensor unit (SU) in a scanning direction (X) and displacement in an approaching direction (Z) towards the associated sample, and

- a sample stage (30) for mounting said associated sample,

wherein the top scanning actuator and the sample stage are arranged for at least two-dimensional (X-Y) scanning of the surface of the sample (S) using the sensor unit (SU, 10) , and wherein the collimator lens (12) is arranged for being adjusted

by said collimator lens actuator along the optical beam path so as to compensate at least for thermal drift in the focus error signal (FES).

2. The AFM according to embodiment 1, wherein compensating for thermal drift in the focus error signal (FES) is performed in a control loop mechanism based on the focus error signal and on displacement, preferably linear displacement, of said collimating lens by said collimator lens actuator.

3. The AFM according to embodiment 2, wherein the AFM is operated at, or near, an optimum sensing point of a corresponding control loop curve.

4. The AFM according to any of embodiments 1-3, wherein the sample stage is mechanically connected to a bottom scanning actuator, more preferably a voice coil motor, said bottom scanning actuator being further arranged for at least one dimensional displacement (Y) for scanning of the surface of the associated sample relative to the sensor unit (SU).

5. The AFM according to embodiment 4, wherein the bottom scanning actuator is further arranged for displacement in the approaching direction (Z) between the associated sample and the sensor unit (SU).

6. The AFM according to any of the preceding embodiments, wherein the AFM tip is arranged for being scanned across the associated sample together with the objective lens in sensor unit (SU).

7. The AFM according to any of embodiments 1-6, wherein the AFM is capable of being operated in a contact mode, a non-contact mode, a tapping mode, a dynamic force mode, a phase imaging mode and/or as an electrostatic force microscopy (EFM).

8. The AFM according to embodiment 1, and optionally embodiment 4, wherein the working range of the top scanning actuator, and optionally the bottom scanning actuator, enable scanning of at least 0.5 mm, preferably

0.75 mm, most preferably at least 1.0 mm, in one or two directions in the plane of performing the AFM scanning.

9. The AFM according to any of the preceding embodiments, wherein the linear scanning speed is at least 50.000 micrometers/second, preferably at least 100.000 micrometers/second, most preferably at least 200.000 micrometers/second.

10. The AFM according to any of the preceding embodiments, wherein the AFM is arranged for dermatological measurements of an associated skin sample.

11. The AFM according to any of the preceding embodiments, wherein the AFM is arranged for scanning of a sample surface under liquid conditions with at least a part of the associated sample being immersed in a liquid, the AFM being capable of adjusting a focal point of the optical path towards the sample by displacing said collimator lens and/or by displacing said objective lens to at least partly compensate for said liquid conditions.

12. A method for operating a HS-AFM and performing measurements of a sample (S), preferably dermatological measurement of a skin sample, the method comprising

- providing a sensor unit (SU, 10) comprising
- a light source (11) capable of emitting a light beam,
- a collimator lens (12) arranged for receiving and collimating said light beam on an optical path towards said associated sample, said collimating lens being mechanically displaceable with a corresponding collimator lens actuator,
- an objective lens (13) receiving and focusing said light beam on an optical path towards said sample, said objective lens being mechanically displaceable with a corresponding objective lens actuator,
- an AFM cantilever with an AFM tip (14) arranged for mechanical engagement with the associated sample, a rear side of the AFM cantilever being arranged for optically reflecting said light beam received from said objective lens in response to the said mechanical engagement, and

- photo detection beams (15) for detecting said reflected light from the AFM cantilever and providing a focus error signal (FES) indicative of said mechanical engagement,

- providing a top scanning actuator (20), preferably a voice coil motor, for at least one dimensional displacement of the sensor unit (SU) in a scanning direction (X) and displacement in an approaching direction (Z) towards the associated sample, and

- providing a sample stage (30) for mounting said associated sample,

wherein the top scanning actuator and the sample stage are mutually arranged for at least two-dimensional (X-Y) scanning of the surface of the sample (S) using the sensor unit (SU, 10), and wherein the collimator lens (12) is adjusted by said collimator lens actuator along the optical beam path so as to compensate at least for thermal drift in the focus error signal (FES).

13. A computer program product being adapted to enable a computer system comprising at least one computer having data storage means in connection therewith to control an AFM according to the embodiment 12.

CLAIMS

1. A high-speed atomic force microscope (AFM, 1) for measurement of an associated sample (S), the AFM comprising:

- a sensor unit (SU, 10) comprising
  - a light source (11) capable of emitting a light beam,
  - a collimator lens (12) arranged for receiving and collimating said light beam on an optical path towards said associated sample, said collimating lens being mechanically displaceable with a corresponding collimator lens actuator,
  - an objective lens (13) receiving and focusing said light beam on an optical path towards said sample, said objective lens being mechanically displaceable with a corresponding objective lens actuator,
  - an AFM cantilever with an AFM tip (14) arranged for mechanical engagement with the associated sample, a rear side of the AFM cantilever being arranged for optically reflecting said light beam received from said objective lens in response to the said mechanical engagement, and
  - photo detection beams (15) for detecting said reflected light from the AFM cantilever and providing a focus error signal (FES) indicative of said mechanical engagement,

- a top scanning actuator (20), preferably a voice coil motor, for at least one dimensional displacement of the sensor unit (SU) in a scanning direction (X) and displacement in an approaching direction (Z) towards the associated sample, and

- a sample stage (30) for mounting said associated sample,

wherein the top scanning actuator and the sample stage are arranged for at least two-dimensional (X-Y) scanning of the surface of the sample (S) using the sensor unit (SU, 10) , and wherein the collimator lens (12) is arranged for being adjusted by said collimator lens actuator along the optical beam path so as to compensate at least for thermal drift in the focus error signal (FES), wherein compensating for

thermal drift in the focus error signal (FES) is performed in a control loop mechanism based on the focus error signal and on displacement, preferably linear displacement, of said collimating lens by said collimator lens actuator.

2. The AFM according to claim 1, wherein the photo detection means (15) comprises a quadrant detector (A, B, C, D) arranged for sensing a focus error signal (FES).

3. The AFM according to claim 2, wherein the quadrant detector is optically arranged relative to an astigmatic lens (ASL) for sensing said reflected light from the cantilever.

4. The AFM according to claim 1, wherein the AFM is operated at, or near, an optimum sensing point of a corresponding control loop curve.

5. The AFM according to any of claims 1-4, wherein the sample stage is mechanically connected to a bottom scanning actuator, more preferably a voice coil motor, said bottom scanning actuator being further arranged for at least one dimensional displacement (Y) for scanning of the surface of the associated sample relative to the sensor unit (SU).

6. The AFM according to claim 5, wherein the bottom scanning actuator is further arranged for displacement in the approaching direction (Z) between the associated sample and the sensor unit (SU).

7. The AFM according to any of the preceding claims, wherein the AFM tip is arranged for being scanned across the associated sample together with the objective lens in sensor unit (SU).

8. The AFM according to any of claims 1-7, wherein the AFM is capable of being operated in a contact mode, a non-contact mode, a tapping mode, a dynamic force mode, a phase imaging mode and/or as an electrostatic force microscopy (EFM).

9. The AFM according to claim 1, and optionally claim 5, wherein the working range of the top scanning actuator, and optionally the bottom scanning actuator, enable scanning of at least 0.5 mm, preferably 0.75 mm, most preferably at least 1.0 mm, in one or two directions in the plane of performing the AFM scanning.

10. The AFM according to any of the preceding claims, wherein the linear scanning speed is at least 50.000 micrometers/second, preferably at least 100.000 micrometers/second, most preferably at least 200.000 micrometers/second.

11. The AFM according to any of the preceding claims, wherein the AFM is arranged for dermatological measurements of an associated skin sample (S).

12. The AFM according to any of the preceding claims, wherein the AFM is arranged for scanning of a sample surface under liquid conditions with at least a part of the associated sample being immersed in a liquid (L), the AFM being capable of adjusting a focal point of the optical path towards the sample by displacing said collimator lens and/or by displacing said objective lens to at least partly compensate for said liquid conditions.

13. A method for operating a HS-AFM (1) and performing measurements of a sample (S), preferably dermatological measurement of a skin sample, the method comprising

- providing a sensor unit (SU, 10) comprising
- a light source (11) capable of emitting a light beam,
- a collimator lens (12) arranged for receiving and collimating said light beam on an optical path towards said associated sample, said collimating lens being mechanically displaceable with a corresponding collimator lens actuator,
- an objective lens (13) receiving and focusing said light beam on an optical path towards said sample, said objective lens being mechanically displaceable with a corresponding objective lens actuator,

- an AFM cantilever with an AFM tip (14) arranged for mechanical engagement with the associated sample, a rear side of the AFM cantilever being arranged for optically reflecting said light beam received from said objective lens in response to the said mechanical engagement, and
- photo detection beams (15) for detecting said reflected light from the AFM cantilever and providing a focus error signal (FES) indicative of said mechanical engagement,

- providing a top scanning actuator (20), preferably a voice coil motor, for at least one dimensional displacement of the sensor unit (SU) in a scanning direction (X) and displacement in an approaching direction (Z) towards the associated sample, and

- providing a sample stage (30) for mounting said associated sample,

wherein the top scanning actuator and the sample stage are mutually arranged for at least two-dimensional (X-Y) scanning of the surface of the sample (S) using the sensor unit (SU, 10), and wherein the collimator lens (12) is adjusted by said collimator lens actuator along the optical beam path so as to compensate at least for thermal drift in the focus error signal (FES), wherein compensating for thermal drift in the focus error signal (FES) is performed in a control loop mechanism based on the focus error signal and on displacement, preferably linear displacement, of said collimating lens by said collimator lens actuator.

14. A computer program product being adapted to enable a computer system comprising at least one computer having data storage means in connection therewith to control an AFM according to the claim 13.

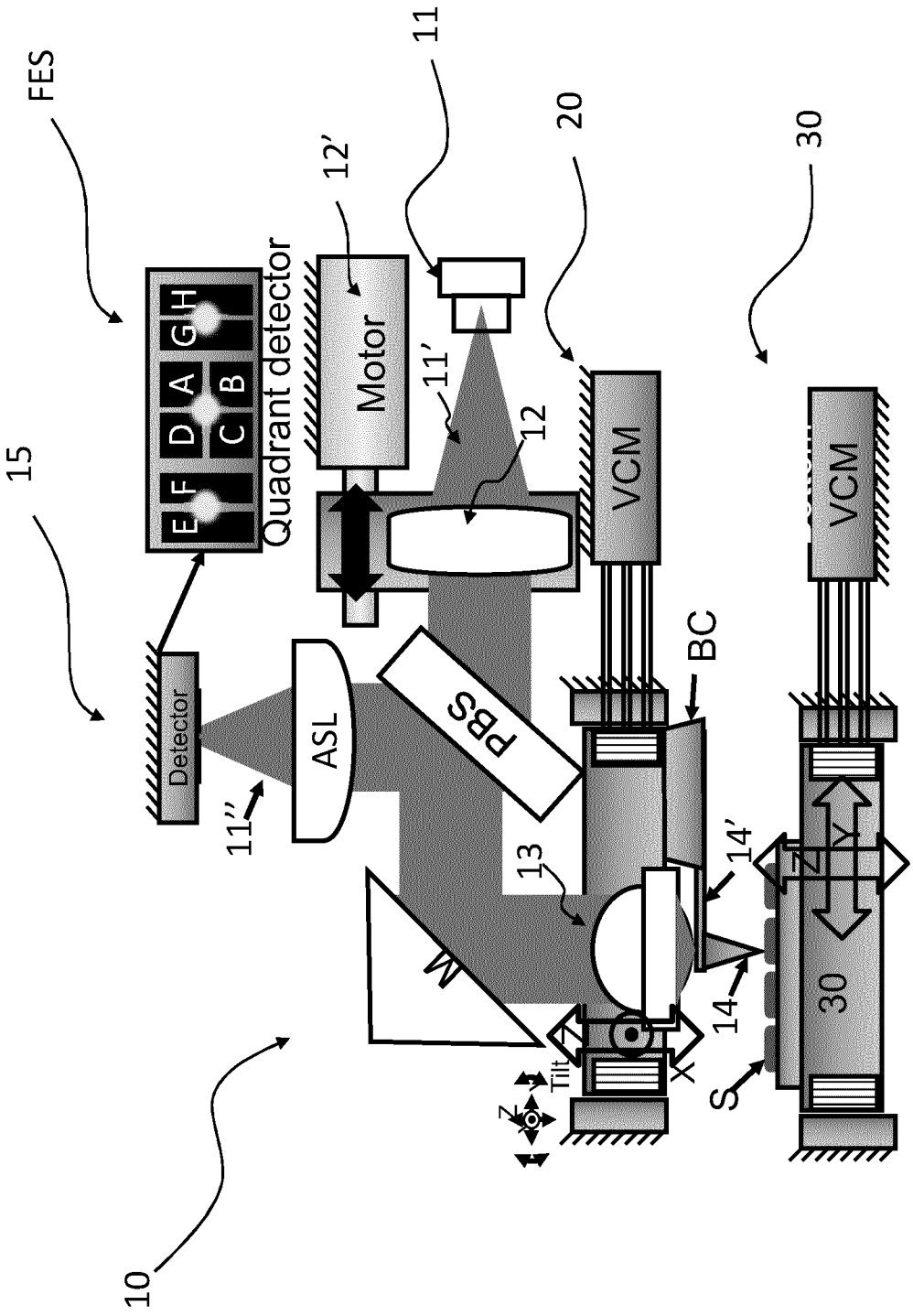
FES
15
10
11
12'
11'
12
20
30
Quadrant detector
E F D A C B G H
Motor
VCM
VCM
Detector
ASL
PBS
BC
11''
13
14'
14
M
Tilt
Z
X
S
Z
Y
30

FIG. 1

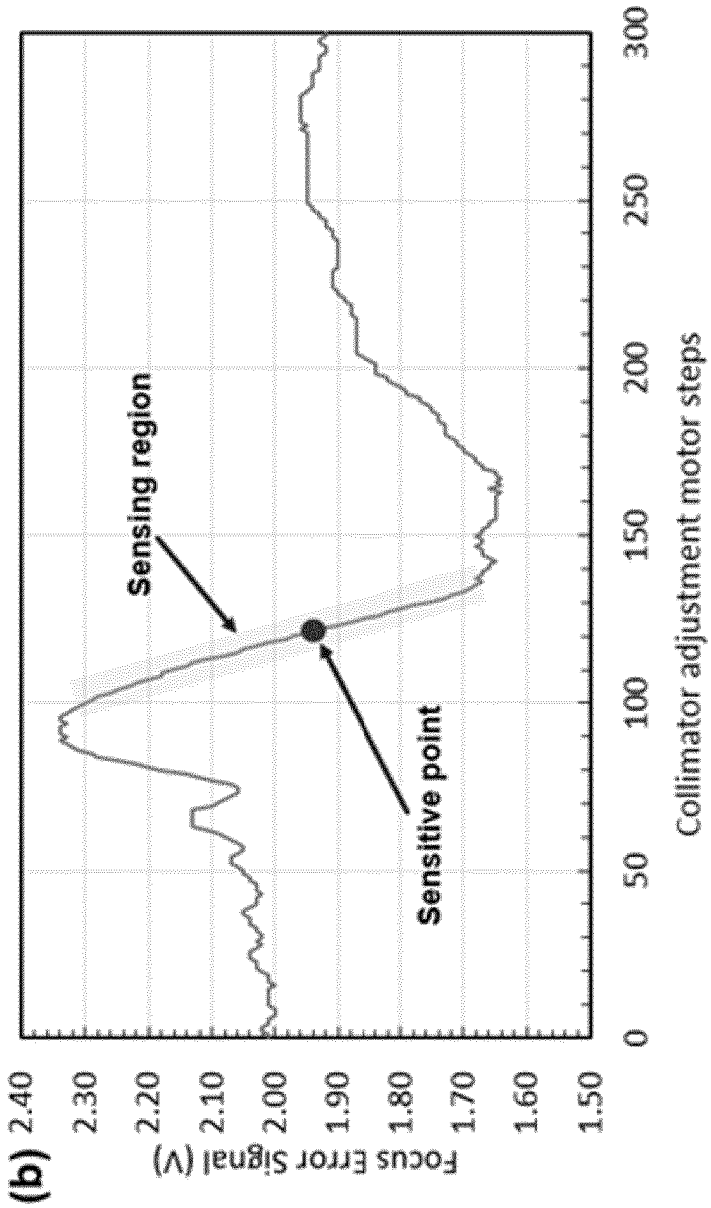
(b)
Focus Error Signal (V)
2.40
2.30
2.20
2.10
2.00
1.90
1.80
1.70
1.60
1.50
0
50
100
150
200
250
300
Collimator adjustment motor steps
Sensing region
Sensitive point

FIG. 2A

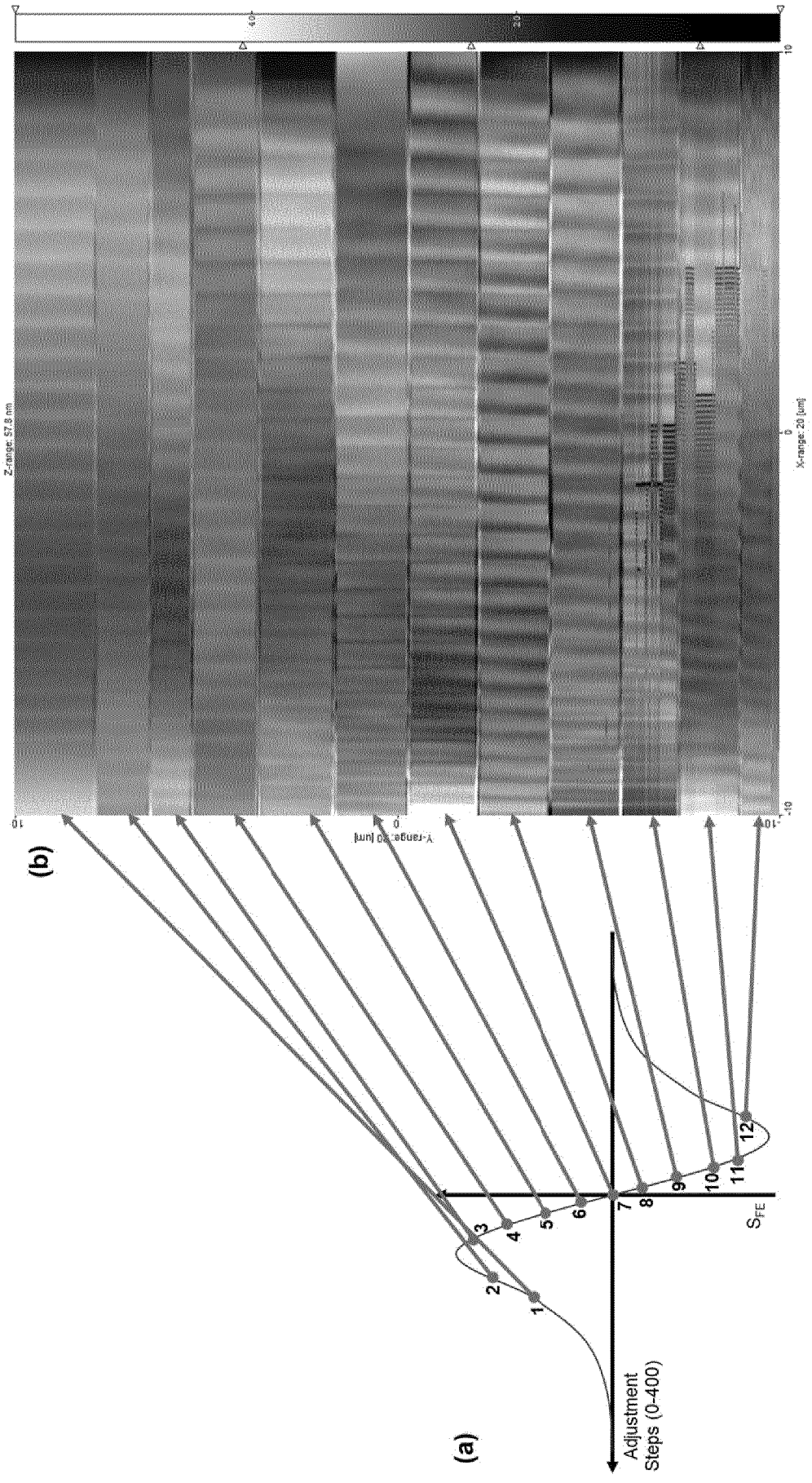
(a)
(b)
Adjustment Steps (0-400)
S_{FE}
1
2
3
4
5
6
7
8
9
10
11
12
Z-range: 57.8 nm
X-range: 20 [um]
Y-range: 20 [um]

FIG. 2B

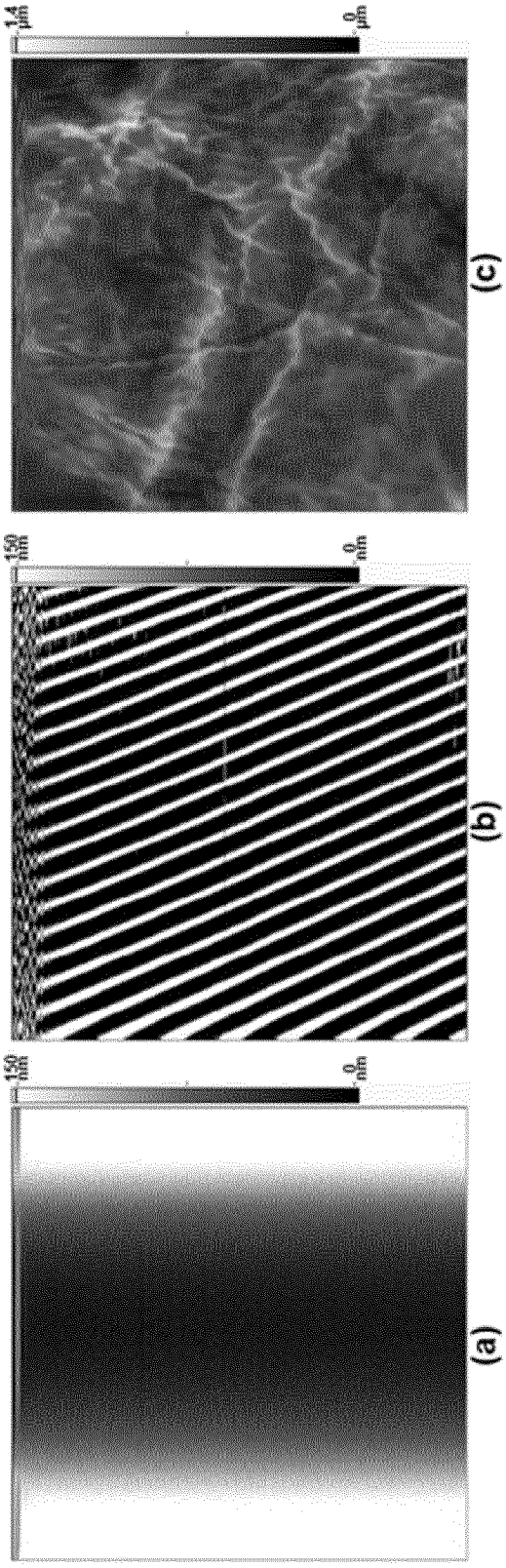

FIG. 3

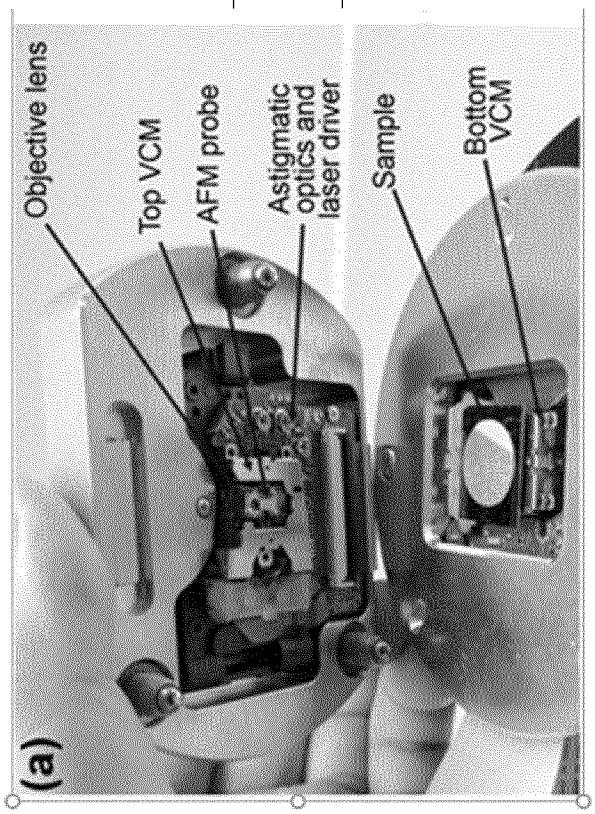
Objective lens
Top VCM
AFM probe
Astigmatic optics and laser driver
Sample
Bottom VCM
(a)

FIG. 4

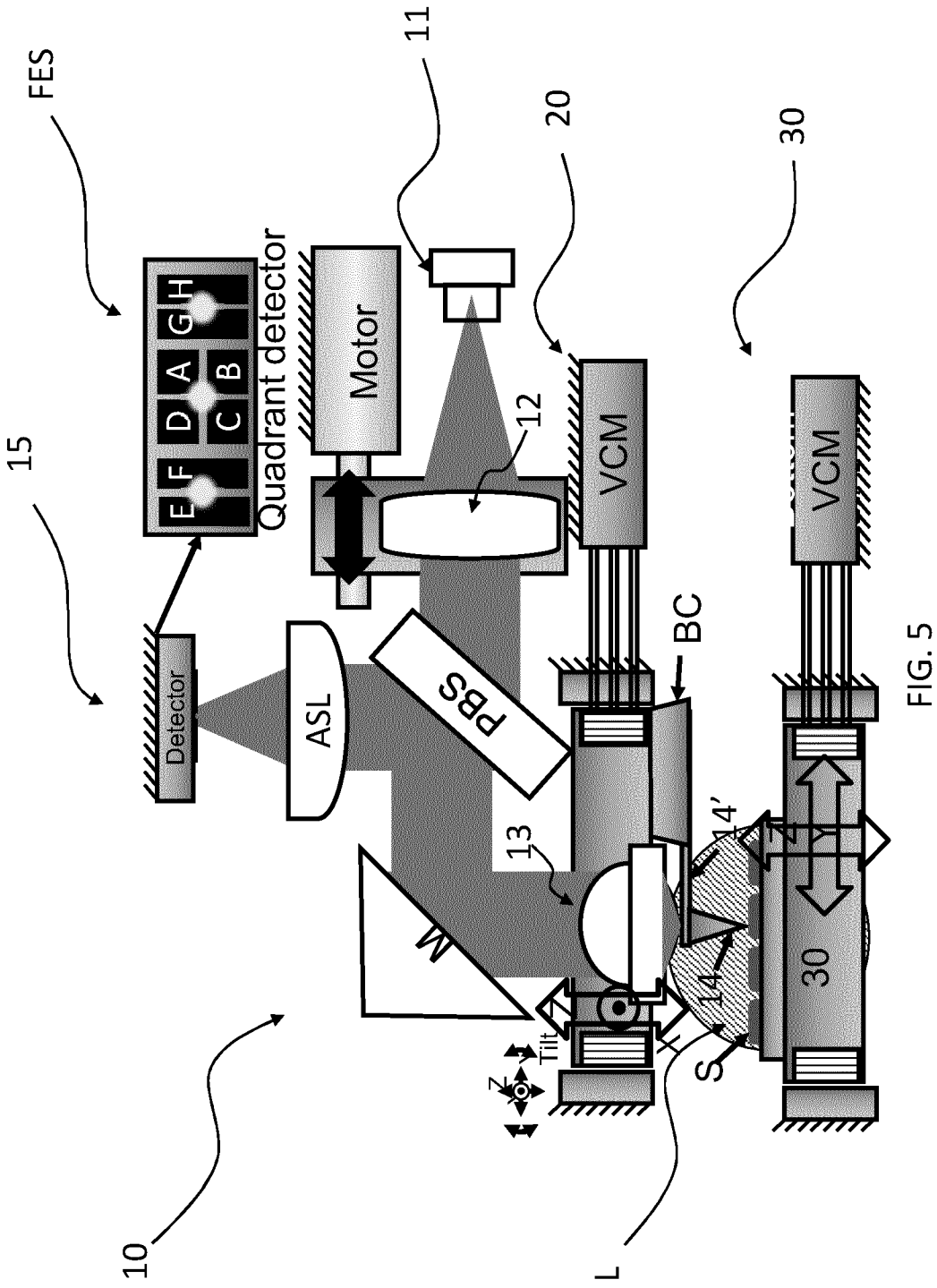
FES
11
20
30
15
Quadrant detector
E F D A G H C B
Motor
12
VCM
VCM
Detector
ASL
PBS
BC
13
14'
14
W
Tilt
Z
S
30
10
L

FIG. 5

1:

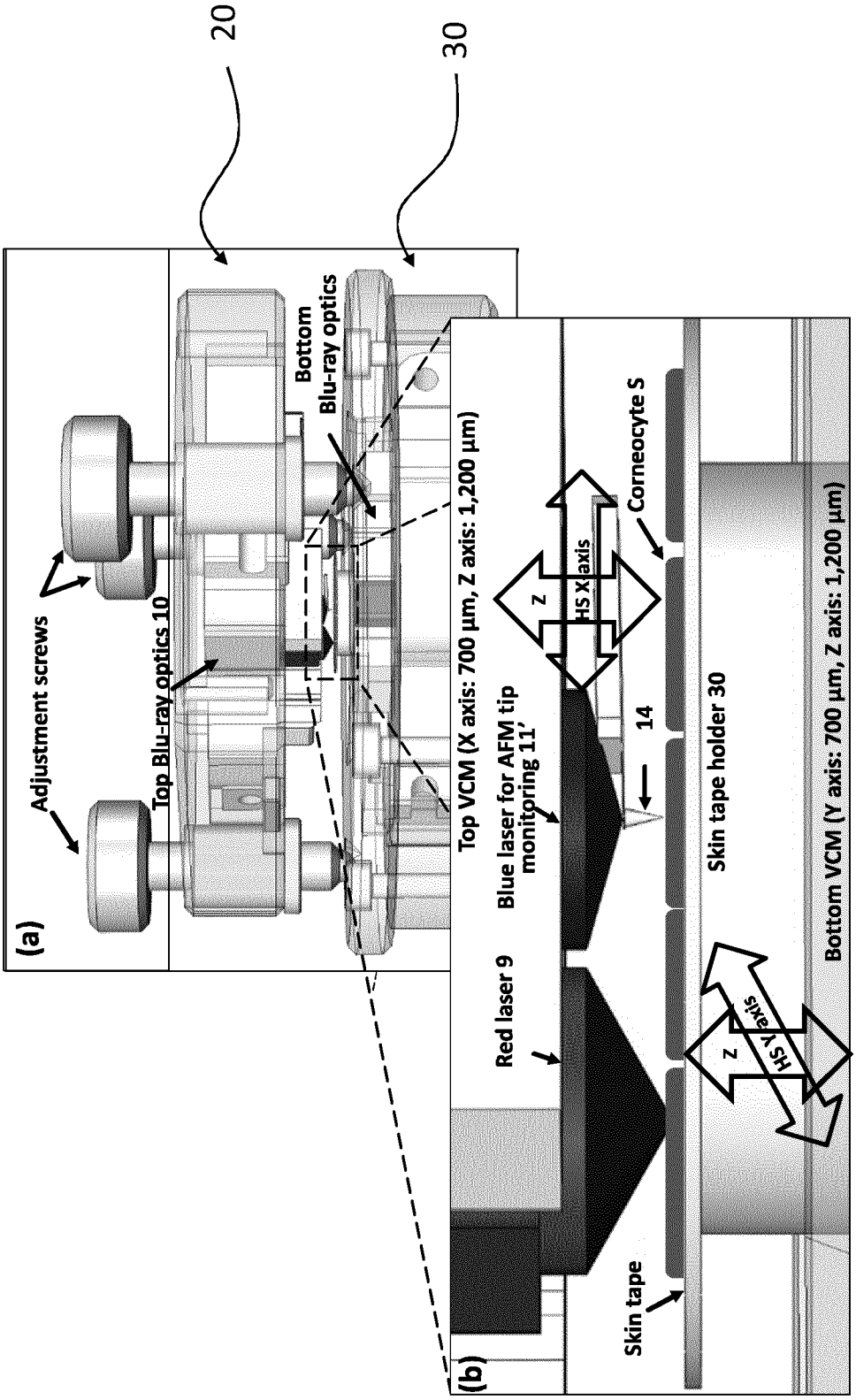
20
30
(a)
Adjustment screws
Top Blu-ray optics 10
Bottom
Blu-ray optics
(b)
Top VCM (X axis: 700 µm, Z axis: 1,200 µm)
Blue laser for AFM tip
monitoring 11'
Red laser 9
Skin tape
Corneocyte S
14
Skin tape holder 30
Bottom VCM (Y axis: 700 µm, Z axis: 1,200 µm)
HS X axis
HS Y axis
Z
Z

FIG. 6

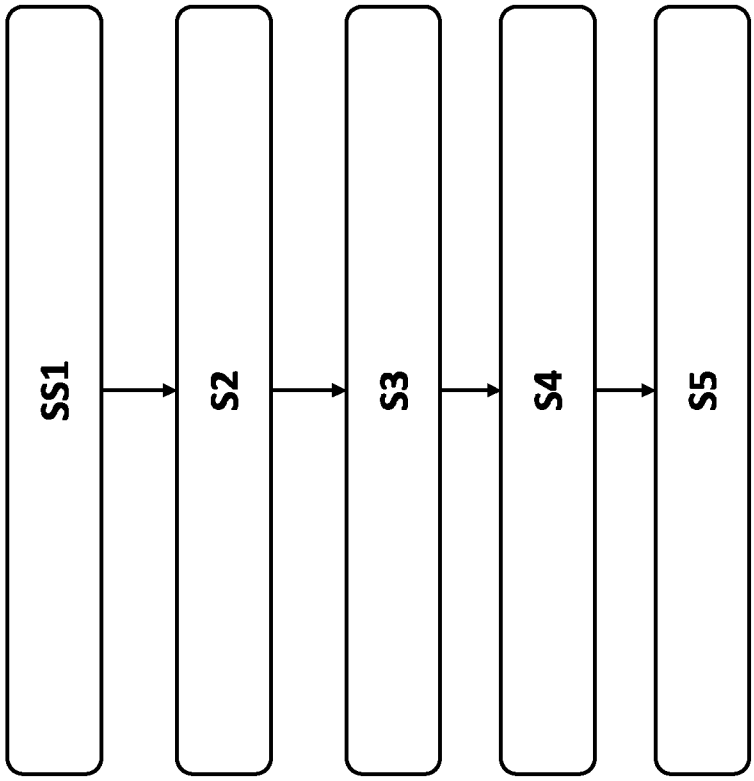

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/060714

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01Q20/02 G01Q70/04
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)
G01Q

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/109803 A1 (OLYMPUS CORP [JP]) 21 June 2018 (2018-06-21)	1,4-14
Y	figures 1-24 paragraph [0005] paragraph [0014] - paragraph [0252] -----	2,3
Y	EN-TE HWU ET AL: "Measurement of Cantilever Displacement Using a Compact Disk/Digital Versatile Disk Pickup Head", JAPANESE JOURNAL OF APPLIED PHYSICS, vol. 45, no. 3B, 1 March 2006 (2006-03-01) , pages 2368-2371, XP055101205, ISSN: 0021-4922, DOI: 10.1143/JJAP.45.2368 figure 2a paragraph [0001] - paragraph [0002] -----	2,3
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[x] Further documents are listed in the continuation of Box C. [x] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2024	16/07/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Polesello, Paolo

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/060714

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2011/098926 A1 (HWANG ING-SHOUH [TW] ET AL) 28 April 2011 (2011-04-28) figure 6 paragraph [0040]	1-13
A	HWU EDWIN EN-TE ET AL: "Hacking CD/DVD/Blu-ray for Biosensing", ACS SENSORS , vol. 3, no. 7 6 July 2018 (2018-07-06), pages 1222-1232, XP093088074, US ISSN: 2379-3694, DOI: 10.1021/acssensors.8b00340 Retrieved from the Internet: URL:http://pubs.acs.org/doi/pdf/10.1021/acssensors.8b00340 cited in the application figures 2, 3, 11 page 2, column 2, paragraph 2 page 6, column 1, paragraph 2	1-13
A	CN 111 458 537 A (UNIV TIANJIN) 28 July 2020 (2020-07-28) figures 1,2	1-13

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/060714

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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US 2011098926 A1	28-04-2011	TW 201115571 A	01-05-2011
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CN 111458537 A	28-07-2020	NONE	