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DESIGN OF PRECISION MEASURING INSTRUMENTS FOR MICROSCOPIC OBJECTS BASED ON VISUAL SENSORS

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Abstract

Optical microscope is used to observe very small object that can not be seen with naked eye. Optical microscope become to be very important tool in various filed from research to industrial field. Beside of observation purpose, optical microscope also widely used as measuring tool.

Development of precision microscopic object measuring tool based on visual sensor is done by adding a camera on ocular lens and driving actuator on the microscope stage. The microscope stage is in precision measured movement, so that larger object can be measured. Microscope stage drive is designed using two stepper motor connected through leadsrew. User interface application was made using Borland Delphi 7. Application converts pixel unit from the camera and step from the motor into micrometer unit.

Test result of measuring tool can measure both objects are objects in the visual area of the camera or larger objects. Range of motion of the microscope table by 50 mm x 40 mm. Actuator of microscope stage has a resolution of 0.2 μm of motion with measured accuracy -0.46% to 0.63%.

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measurement tool
microscope
motorized stage

1. Introduction

Optical microscopes are important tools in today's research field. They are widely used in hospitals, laboratories, and educational institutions for various purposes. Not only in biology, microscopes are also used in materials science and as inspection tools in industries such as electronics, printing, textiles, and so on.

Microscopes have the basic function of viewing objects that are very small in size. The addition of various features to microscopes, such as cameras and microdrives, can increase their applications and usefulness. By combining mechanical and optical systems, microscopes can even be used as precision measuring instruments. Some research or industrial inspection objects require that the object not be touched directly, necessitating visual measurement, which can be assisted by a microscope.

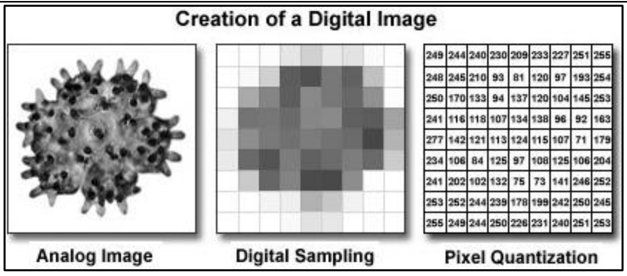
Microscope objects whose entire cross-section can be viewed with a microscope can be measured directly visually, but if the object is longer than the microscope's visual area, an additional drive is needed that can shift the object precisely and measurably

A low-cost prototype microscope with a motorized drive has been developed by Vargas, C. et al. for the purpose of automating microscope imaging. The microscope used is a CARL ZEISS Axiostar plus trinocular microscope, with a drive using a stepper motor with 200 steps per revolution. By changing the voltage configuration on the stepper motor, the motor can produce 102400 micro steps ( ), thereby changing the actuator accuracy from 25.4 μm to 0.00025 μm. With further modifications, it can produce an accuracy of 1 μm (Vargas, 2006). The micro-drive system can be applied to various types of microscopes without needing to modify the microscope itself. The application is designed solely to control movement and does not create a more specialized application.

Xu Ma et al. (2009) have created a microdrive application for microscopes that can be used to shift specimens. The drive can remember the position previously set by the doctor, making subsequent examinations easier. The drive system is controlled by two DSP (Digital Signal Processing) controllers connected by CAN (Controller Area Network). The results of the drive system testing showed a repeatability error of 1 μm.

Vision Sensor

An image is a two-dimensional representation of a three-dimensional physical form, which can take various forms. These range from black and white images in a still photograph to moving color images, better known as videos. A digital image is a continuous image that has been discretized, both in terms of spatial coordinates and brightness, through a process of sampling and quantization (Figure1). Sampling is the process of digitizing object coordinates into raster coordinates. This process determines the image size, e.g., 10 x 10 pixels. Quantization is the process of digitizing the intensity of the object signal at the sampled pixel coordinates, or in other words, assigning a value to that pixel.



249	244	240	230	209	233	227	251	255
248	245	210	93	81	120	97	193	254
250	170	133	94	137	120	104	145	253
241	116	118	107	134	138	96	92	163
277	142	121	113	124	115	107	71	179
234	106	84	125	97	108	125	106	204
241	202	102	132	75	73	141	246	252
253	252	244	239	178	199	242	250	245
255	249	244	250	226	231	240	251	253

Figure 1. Image Digitization Process
(Spring, R dkk, http://learn.hamamatsu.com, accessed June 20, 2012)

Stepper Motor

A stepper motor is an electromechanical device that converts electrical pulses into discrete mechanical movements. The stepper motor shaft rotates in discrete steps when electrical pulses are applied in the correct sequence. The rotation of the motor is related to its pulse input. The sequence of input pulses is directly related to the direction of rotation of the motor shaft. The speed of rotation of the motor shaft is determined by the frequency of the input pulses, and the length of rotation is directly related to the number of input pulses applied.

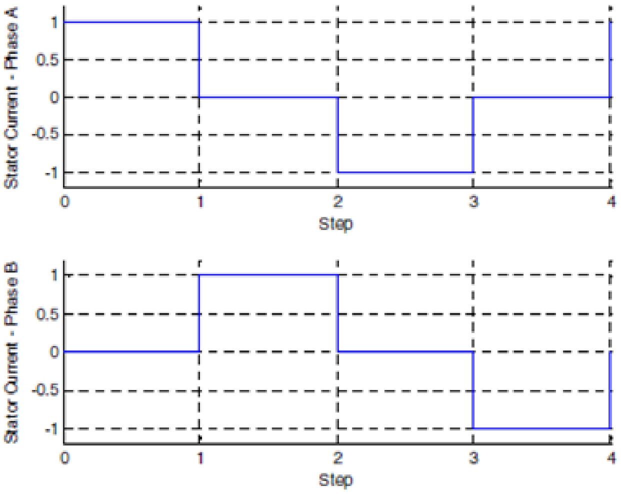
The main advantages of using stepper motors are that they are easy to control and can be controlled in an "open loop" manner, eliminating the need for feedback on the motor's position. This type of control eliminates the need for position sensors, which are usually expensive. The position of a stepper motor can be easily determined through the step input provided.

There are three basic types of stepper motors, namely:

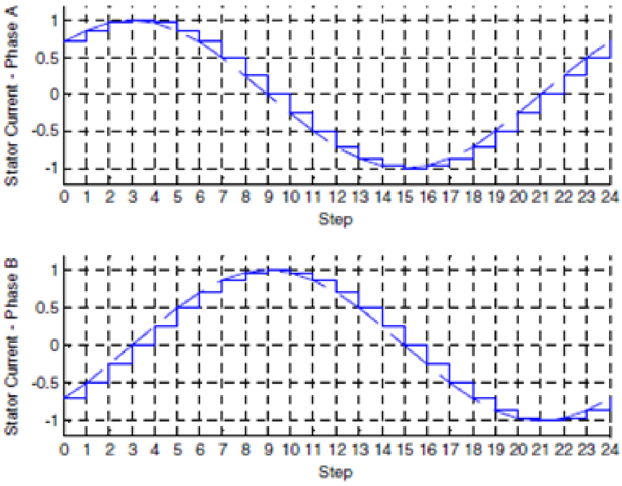
- *Variable-Reluctance*
- *Permanent-Magnet*
- *Hybrid*

Common control modes used in stepper motors:

a. Wave control 1 fase *on*)
b. Full step control (2 fase *on*)
c. Half step control(1 dan 2 fase *on*)
d. Miicro step (continously varying voltage)



Current waveforms in the common driving mode.



Current waveforms in the microstepping driving mode.

Figure 2. Normal and Micro Step Control Modes (Xu Ma, 2009)

2. Methodology

The device created is a modification of a binocular microscope with the addition of a camera and a microdrive on the specimen stage, as shown inFigure3 . The camera is used to capture images of the microscope object into images in the form of a series of pixel digits, while the micro-drive is used to move the object in a measured manner. The combination of digital images from the camera and the micro-drive is used to measure the object. The device consists of two main parts: hardware and software.

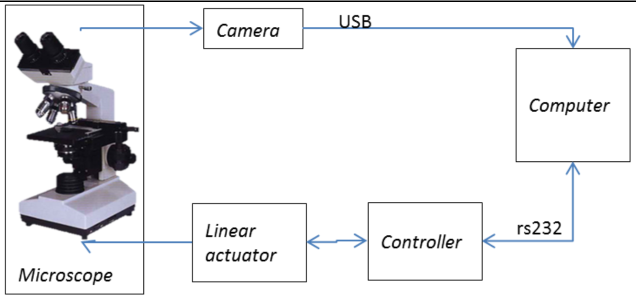


Figure3 . Device Design

The hardware in this study is divided into two parts:
1. optical devices consisting of a camera and lighting system
2. microdrivers

Microscope Camera

In principle, this camera replaces the eyepiece lens and the human eye, so in this design, the camera is placed in the position of the eyepiece lens. In this study, a camera with a resolution of 1024 x 768 with a USB connection was used for data communication between the camera and the computer. A USB CMOS camera was chosen because of its ease of communication with computers and the relatively low price of CMOS compared to CCD cameras. The camera is designed to be securely mounted on the microscope and to capture clear microscope images. The structure of the microscope camera is shown atFigure4 where the CMOS sensor replaces the human eye.

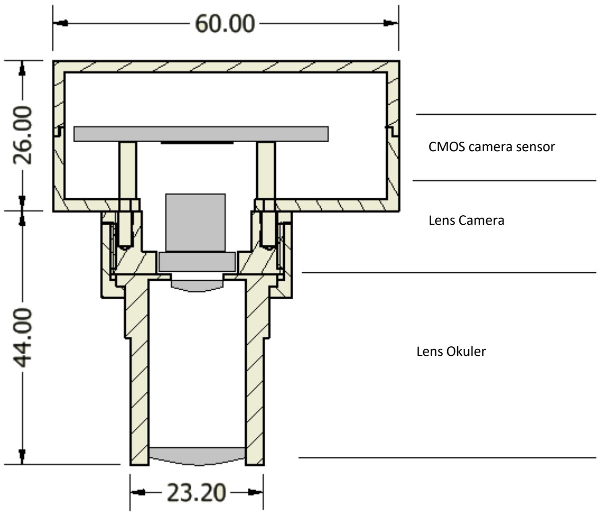


Figure 4. Microscope Camera Structure

Specimen Stage Microdrive

The micro drive mechanically connects the stepper motor to the specimen stage slider, used to move the object being observed under the microscope. The micro drive is designed to move in two dimensions. It uses two hybrid stepper motors and is controlled using microsteps. Hybrid stepper motors were chosen because they have the most steps per revolution compared to other types of stepper motors.

Linear actuators are used to convert rotational motion into linear motion. The linear actuator used is a leadscrew type due to its relatively low price, even though leadscrews have a high error rate compared to ballscrews. The screw shaft and nut in this linear actuator have a loose fit, which must be addressed to prevent measurement errors, especially when the motor moves in the opposite direction. When the motor rotates in the opposite direction from the previous rotation, the nut does not move immediately due to the looseness. This type of error is called hysteresis error and is common in translation systems that use leadscrews. The error is overcome by applying an initial load to the nut on one side only using a spring force, as shown in Figure5. This system is also called anti-backslash.

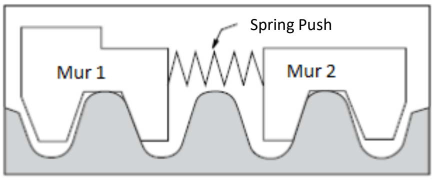


Figure5 . Rotation in the Motor is Converted into Linear Motion, the Nut is Pushed with a Spring to One Side to Prevent Errors

A single step on this motor has a displacement of 0.0032 mm, or more precisely 0.003175 mm. With the target of producing a movement accuracy of 1 µm per step, the stepper motor must be controlled using micro steps. A 16 microstep divider was selected, resulting in an accuracy of 0.1984375 µm.

The manual slider on the microscope stage design is replaced by a motor. The motor is mounted on the microscope stage with an aluminum structure design as shown inFigure6 . The aluminum frame structure is machined using a CNC machine.

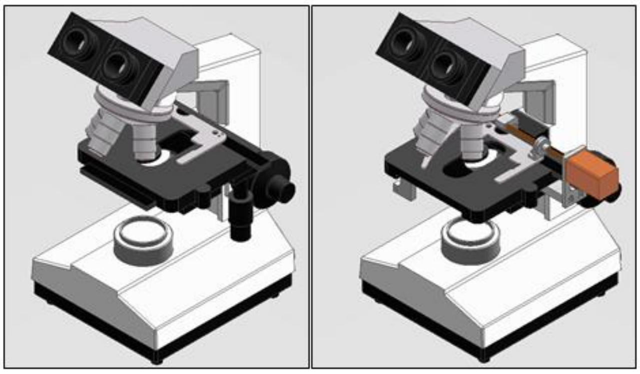


Figure 6. Microspe Before and After Stage Modification

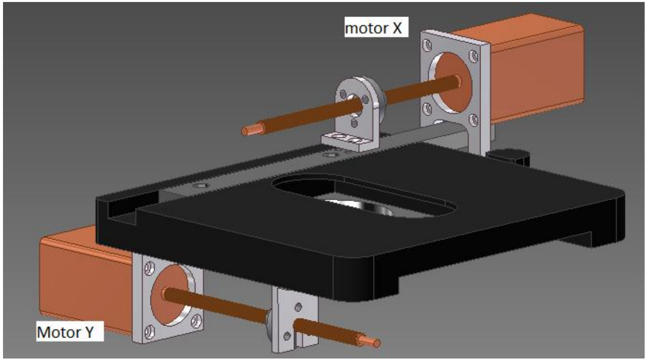


Figure 7. Micro-Diver Structure on the Microscope Stage

Motor Control

The stepper motor is designed to be controlled using a computer. Several steps are required to enable computer control of the motor. The motor is first connected to a microstep driver, then to a microcontroller. The microcontroller is connected to the computer via a serial port, or alternatively via a USB-to-serial port converter. The complete configuration can be viewed at Figure8 .

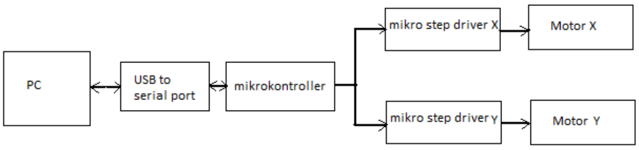


Figure 2. Konfigurasi Piranti untuk Pengandalian Motor Langkah Menggunakan Komputer

Limit Switch

The stepper motor is designed to be controlled using a computer. Several steps are required to enable computer control of the motor. The motor is first connected to a microstep driver, then to a microcontroller. The microcontroller is connected to the computer via a serial port, or alternatively via a USB-to-serial port converter. The complete configuration can be viewed at Figure8 .

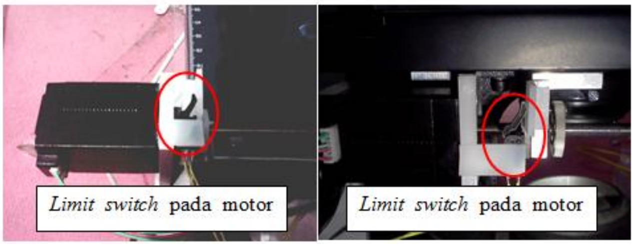


Figure 9 . Limit Switch as Zero Point Reference and Motor

Safety DeviceInitialization is performed when the device is first turned on, as shown in the flowchart at Figure 10. . The microcontroller checks the status of the limit switch. If the limit switch is connected, it means the motor is already at the zero position. If the limit switch is not yet connected, the motor moves backward until the limit switch is connected. The motor stops and is declared as the motor's zero point. The motor status is set to "ready for use."

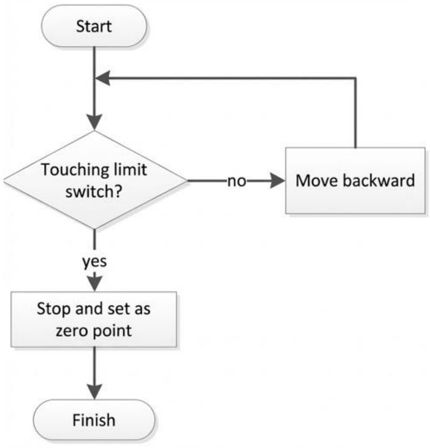


Figure 10 . Motor Initialization Flowchart Steps

Software Design for Object Dimension Measurement Interface

To meet the requirements for a measuring tool, the software must be developed based on the following criteria:

1. have a microscope object image display
2. the position of the object is known from the position of the computer cursor when clicked on the image of the object being measured.
3. Object dimension measurements are performed directly for objects that are fully visible on the image display screen.
4. Object dimensions are measured in pixels and then converted to micrometers.
5. Objects larger than the image display can be measured by moving the motorized microscope stage using the software.
6. Object dimension measurements include:
   a. Distance between two points
   b. Circular shape approximation
   c. Distance between the centers of two circles, and

d. Measurement of arbitrary shapes.

Knowing the Position of the Motor

The position of both the X and Y stepper motors on the microscope stage must be known in order to be used as a basis for measurement. The number of steps is converted to micrometers using the equation:

$$Mikrometer = \frac{step}{5} \times 0.9921875 \qquad (1)$$

Due to hysteresis error in the translation, the motor position status is adjusted as shown in the flowchartFigure11 . During microscope stage initialization, the motor moves backward so that when the motor moves forward, the step position status is reduced by the hysteresis error value.

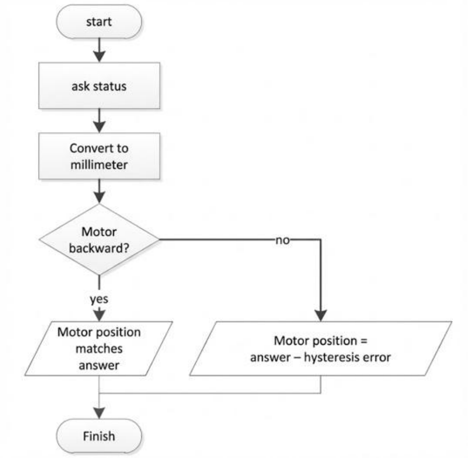


Figure 11 . Obtaining Motor Position

Pixel Calibration

Pixel calibration is performed to convert the pixel units from the camera image into micrometer units. Different X and Y pixel calibrations are required, so calibration is also performed twice for each lens type using the following equations:

$$X\ calibration = \frac{actual\ micron\ value}{selected\ pixels} \times camera\ image\ pixel\ width \qquad (2)$$

$$Y\ calibrarion = \frac{actual\ micron\ value}{selected\ pixels} \times camera\ image\ pixel\ height \qquad (3)$$

3. Results and Discussion

Hardware Design Results

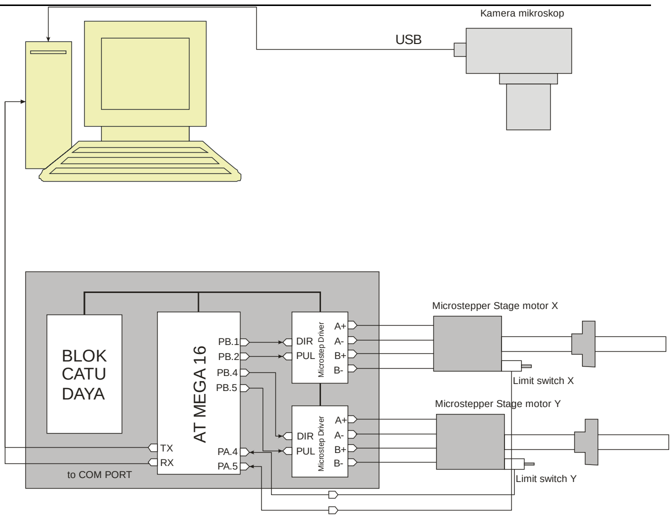


Figure 12 . Schematic Diagram of the Measurement Device Hardware

Stepper Motor Speed

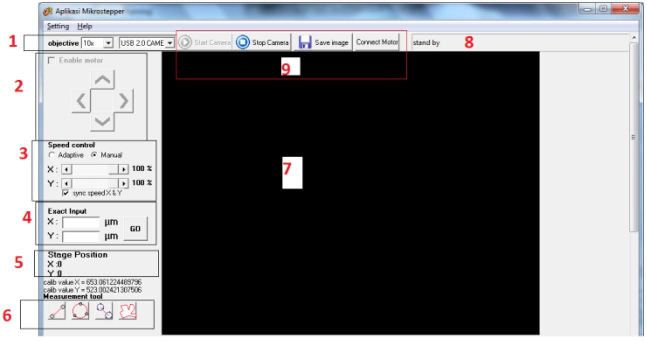
Test results by varying the pulse width as follows:

Table1. Motor Input Pulse

Lebar pulsa	Respon motor
47 μs	gagal
54 μs	OK

Based on the test results, the motor is capable of operating with an input pulse of 54 µs. With an input pulse of 54 µs, the table can move at a speed of 3.7 mm/s. The speed of the microscope table with this micro drive can be adjusted by providing a multiple value to the input pulse.

Software Design Results



objective 10x

USB 2.0 CAME

stand by

X : 100 %
Y : 100 %
☑ sync speed X & Y

X : µm
Y : µm

Stage Position
X :0
Y :0
calib value X = 653.061224489796
calib value Y = 523.002421307506

Figure 13 . Microstepper Application Display

The software application developed using Delphi for measurement tasks has an interface as shown inFigure13 and is named "Microstepper Application." As seen in the figure, the microstepper application consists of the following components:

1. Settings panel, used to select the settings for the objective lens, camera, and com port.
2. Microscope stage control panel, used to control the position of the microscope stage with 4 arrow keys. The stage will move according to the direction of the button pressed.

3. Speed control panel, used to control the speed of the stage movement. There is a manual speed control and also an adaptive control. If the adaptive control is activated, the stage initially moves slowly and then moves faster.
4. Exact input panel, where inputs are entered in microns. If the input is positive, the stage moves forward, and if the input is negative, the motor moves backward.
5. Table position information panel, displays the coordinate position of the microscope table.
6. Measurement mode panel, used to select the desired measurement mode.
7. Microscope object image display panel, which displays the microscope object image and the measurement area.
8. Software status panel
9. Motor and camera control panel.

Pixel calibration is the process of converting image units in pixels to micrometer units. Calibration is performed twice for each type of objective lens used, namely horizontal pixel calibration and vertical image calibration.

Table2. Pixel Calibration Results

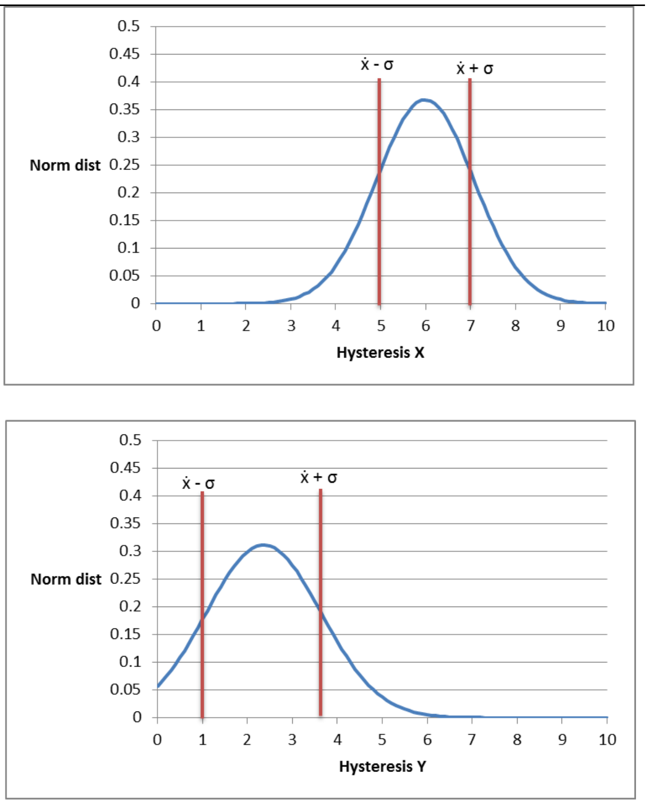
Lens	X Calibration (Horizontal)	Y Calibration (vertical)
4X	1581.02766798419	1263.15789473684
10X	653.59477124183	524.017467248908
40X	-	-
100X	-	-

Hysteresis Error Test

The results of the hysteresis error test for this microscope table are as follows:

	X	Y
Histeresis	5.99	2.36
St-dev (σ)	1.09	1.28

The magnitude of the deviation and its probability can be seen in the normal distribution graph at Figure14 .



Fifure 3. Normal Distribution of Hysteresis Error Values

If the diagonal deviation of this measuring instrument is calculated based on the standard deviation (σ) of X and Y, the deviation is 1.68 µm.

Microscope Stage Drive Range

The microscope stage has a maximum movement range of 5 cm on the X-axis and 4.5 cm on the Y-axis. The microscope stage drive with a stepper motor is designed with a movement range of 4.5 cm on the X-axis and 4 cm on the Y-axis. Its movement range is slightly smaller than the maximum range but is safer.

Accuracy Test

The accuracy test was conducted by measuring test samples with varying lengths using a micrometer objective, then comparing the measurement results with the actual size reference. The variations ranged from 100 µm to 1000 µm. The accuracy value is the largest negative and positive error value. The accuracy test results are shown at Table3 . Based on the test results, the accuracy value of the measuring instrument is between -0.46% and 0.63%.

Table3 . Accuracy Test Results for Measurement

Test Sample	Result 1	Result 2	Result 3	error		
				1	2	3
100	99,9	99,9	100,5	-0,10 %	-0,10 %	0,50 %
200	199,8	199,8	199,8	-0,10 %	-0,10 %	-0,10 %

300	299,8	300,3	299,8	-0,07 %	0,10 %	-0,07 %
400	400,9	399,6	399,6	0,22 %	-0,10 %	-0,10 %
500	501,4	501,4	500,8	0,28 %	0,28 %	0,16 %
600	600,7	598,1	597,8	0,12 %	-0,32 %	-0,37 %
700	697,9	696,8	698,0	-0,30 %	**-0,46 %**	-0,29 %
800	805,0	804,0	800,5	**0,63 %**	0,50 %	0,06 %
900	901,6	901,9	902,5	0,18 %	0,21 %	0,28 %
1000	999,9	1003,1	999,0	-0,01 %	0,31 %	-0,10 %

4. Conclusion

1. The combination of a camera and a table with a microdrive on the specimen table can be used as a precise and accurate measuring device.
2. The motion resolution of the measuring device is 0.2 µm.
3. The hysteresis error of the specimen stage drive is 5.99 µm for the X-axis and 2.36 µm for the Y-axis, with repeatability of 2.17 µm for the X-axis and 2.56 µm for the Y-axis.
4. The accuracy of the measuring device is between 0.46% and 0.63%.

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