ELEMENTAL TECHNOLOGIES FOR GENOME-BASED DRUG DISCOVERY TEST SYSTEM USING CULTURED LIVE CELLS

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Since the decoding of the human genome, "Genome-based Drug Discovery" based on molecular-level analysis of deceased people has been gathering considerable expectation. However, the currently low chance of proceeding to clinical trials poses as the topmost task in the development of new drugs. Given this circumstance, we have started to develop a prototype of a drug discovery test system to offer solutions for reducing development time and costs for the genome-based drug discovery process. This system administers chemical compounds that serve as potential candidate drugs into live cells, which are the most basic components of all living organisms, records the changes in the amount and/or localization of target molecules inside cells with our highly sensitive CCD camera, and processes and quantifies the captured high-resolution image data. This screening method enables the drug efficacy and adverse drug reaction of the candidate chemicals to be verified and the candidate drugs to be confirmed in live cells. This paper describes on a typical elemental technology that composes the Test System.

INTRODUCTION

Recent developments in molecular biology such as genomics and proteomics and/or life science such new disciplines as system biology and chemical biology have been continuously revealing the factors or causes that trigger various human diseases. Particularly, since the successful decoding of the human genome, great expectations have been placed on the "genomic drug discovery", a process to unravel the mechanisms of diseases at the molecular level and to develop drugs based on the resulting findings. Expectations have also been put on the development of new effective drugs for diseases that have been difficult to treat with conventional drugs or therapies. Currently, however, only a small proportion of these newly developed drugs have passed the clinical tests, posing a serious challenge. To break through this problem, the importance of testing at the cell level has begun to be more recognized.

Under these backgrounds, we have designed and manufactured a prototype of the genomic drug test support system that helps to reduce the development time for new genomic drugs as well as the development cost (Figure 1). This prototype has been built by fully leveraging our accumulated technologies including mechatronics, environmental control

Figure 1 External View of the Prototype

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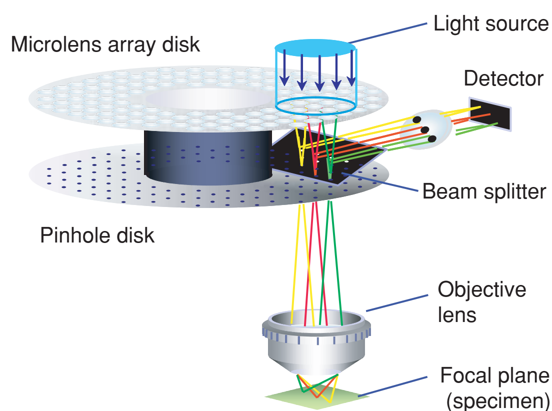

Figure 2 Configuration and Principle of CSU

technology, advanced image processing technology, and optics design technology that has been used to create our confocal microscope. This paper reports on the major elemental technologies used for the development of the prototype.

FEATURES OF THE TEST SYSTEM

(1) The sensing component of the manufactured test system employs the confocal scanner with the Nipkow disk (consisting of microlens and pinhole disks), which Yokogawa has been marketing and selling for the purpose of biological research (product name: CSU). Figure 2 shows the configuration and principle of the scanner. For more details, refer to other paper in this special issue [1].

This sensing method has the following features:

① The confocal imaging technique allows for the measurement of cellular cross-sections. Intracellular granules can be measured with high precision.

② Three-dimensional observations by z-axis scanning are possible. The structure of neurites can also be viewed.

③ Our sensor provides a much less photobleaching property, about 1/15 that of the equivalent product from other companies. This feature allows for continuous long-time observations[2].

④ Our sensor provides an extremely high imaging speed, 1000 times faster than the product from other companies, enabling the observation of the fast Ca2+ spark.

(2) Use of the super high-sensitivity, electronic multiplication CCD camera allows the user to capture images with a high S/N ratio.

(3) The high-performance spectroscopic system provides the capability of simultaneous multicolor image formation.

(4) The carrying system is designed to meet industrial applications like factory automation, where 24-hour operation is required. As a result, the system itself provides high levels of speed, precision and reliability, and a broad application range from high-throughput drug development to general research activities.

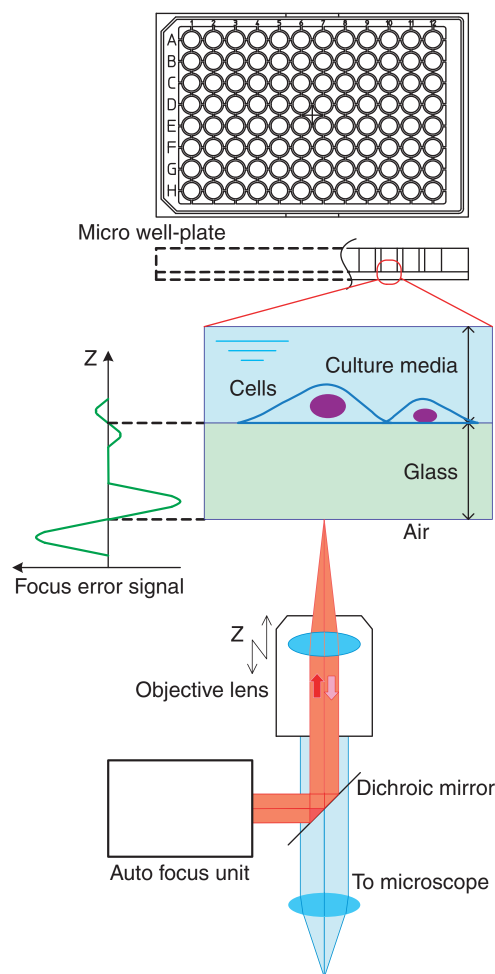

Figure 3 Principle of Auto-Focus

ELEMENTAL TECHNOLOGIES USED IN THE PROTOTYPE OF THE TEST SYSTEM

The manufactured test system incorporates many elemental technologies. Of those, three major technologies are reported here.

Auto focus unit

The auto focus unit is a device that detects the bottom surface (e.g., bottom plane) of the glass at the bottom of the micro well-plate by utilizing an optical reflection caused by the refractive index difference between the air and the glass. The purpose of this unit is to obtain the position of the observation plane accurately and automatically. Figure 3 shows the principle of the auto focus unit. It uses a long wavelength infrared light (780 nm) as a focal point detection light, in order to reduce possible damage to the specimen as well as to distinguish the reflected detection light from the fluorescent signal emitted from the specimen. The auto focus mechanism of the unit collimates the infrared light irradiated from a laser diode into a parallel light beam, which enters and passes through the objective lens. This light reaches the bottom surface of the glass at the bottom of the micro well-

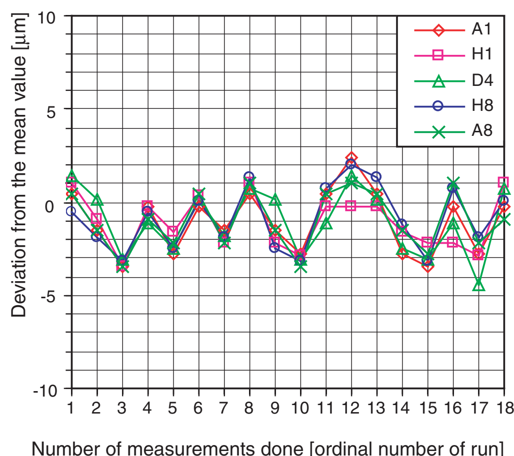

Figure 4 Position Reproducibility at the XY Stage
(A1, H1, D4, H8 and A8 are addresses that define a location of each well of the micro well-plate)

plate, and about 4% of the light is reflected from the glass surface. Then the light enters the objective lens before returning back through the reverse path. Part of the returning light is reflected by a beam splitter, and this reflected light is detected by a special photo sensor as a focus error signal.

The unique aspect of this unit is that it detects the position of a focal point by measuring a change in aberration of the returning light of the focal point detection beam. When the focal plane of the objective lens is above or below the bottom surface of the glass of the micro well-plate, the shape of the aberration takes an orthogonal form. On the other hand, when the focal plane is located at the bottom surface of the glass, the shape of the aberration assumes a symmetric form. In other words, if a sensor is used to measure information provided by such aberration shapes or curves, the sensor output (focus error signal) will decrease gradually as the focal plane of the objective lens is moved towards the bottom surface of the glass. In the meantime, the sensor output will reach zero when the focal point of the objective lens is located exactly at the bottom surface of the glass. Furthermore, the sensor output will increase gradually from Zero as the focal point of the objective lens is moved away from the bottom surface of the glass.

Figure 3 illustrates the relationship between the position of the objective lens's focal plane and the magnitude of focus error signal.

When an actual observation is performed on the test system, first the auto focus unit is used to detect the bottom plane of the glass for a well containing a specimen as described above. Then a certain offset or distance from the bottom plane of the glass to the specimen is added to the bottom plane to obtain the observation plane actually used for imaging.

The auto focus unit offers a high-precision focal point detection capability of 0.3 µm, which is higher than the resolution in the direction of confocal optical axis, enabling precise, error-free image acquisition.

Robotics

The manufactured drug test system incorporates a total of 20 or more motors, which are used for the handling mechanism for micro well-plate positioning and the drive mechanism for objective lens control and light path switching. In drug screening processes, usually a maximum of several hundred well plates are processed per run. To meet this situation, the system requires both high-speed operating capability and high durability. The drug test system we have developed has realized reliability that ensures continuous operation, which has been achieved based on our know-how acquired through experience in the production of semiconductor-related manufacturing equipment.

Cancer researchers often analyze and track changes in cells by observing the same specimen for several days on the time lapsing basis. In these experiments, the well plates that have been observed are usually placed back to the incubator and then will be observed again after a specific length of time. This sequence is repeated many times. For this reason, highly precise position reproducibility is demanded for the carrying system in order to ensure an accurate observation of the same cells.

In cell tracking, if the positional difference between the last observation (imaging) and the next observation is about the same as the size of the target specimen (cell), it is easy to track the target by performing image processing. Under this consideration, the carrying system is designed to provide a precision of 10 µm, approximately equal to the size of the nuclear of a HeLa cell which is commonly used in drug development.

Figure 4 shows the position reproducibility at the XY stage obtained from the test system we have developed. The results have been obtained as follows: the stage of a 96-hole well plate is moved so that the A1, H1, D4, H8 and A8 holes (A1, H1, H8 and A8 are holes at four corners of the well plate, and D4 is a hole at the center) are positioned at a specimen observation location in this order, then the position of each hole is computed through image processing. This sequence has been repeated many times. From the results, it was found that the position reproducibility of the stage falls within the designed range of ±10 µm.

Divided jet injection dispenser

Technique of divided injection is important for performing tasks such as the dilution of compound and the examination of the medicine's effect on cells. There may be a case in which our test system is used for live-cell observations where a reagent is dropped onto the cells and the reaction occurring in those cells is studied. To serve such purposes, we have developed a high-speed dispenser that can provide divided injection of solution with high precision and without being affected by the characteristics of such solution.

One conventional method is to execute divided injection by pushing the piston in a syringe to inject the solution. This technique has a drawback that a small amount of the injected solution remains inside the tip of the needle because the injection speed is slow. As a result, this type of method cannot provide accurate divided injection, or will completely fail to perform divided injection when the amount of solution to be delivered is very small.

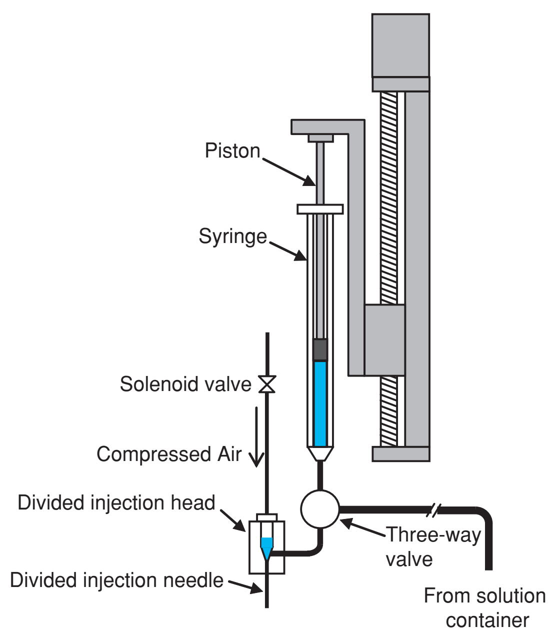

Figure 5 Basic Structure of the Dispenser

Another conventional approach includes the use of a high-speed solenoid valve, which is installed at the bottom of an injection needle, in order to deliver a small divided amount of pressurized solution contained in a tube by opening and closing the solenoid valve. However, this divided injection technique has a problem. That is, the amount of delivered solution is controlled by the level of pressure produced by the pressure pump and the time length during which the valve is opened. Therefore, the viscosity of solution determines the actual length of time required for the solution to pass through the solenoid and through the narrow tube, and thus the amount of solution delivered. In other words, the amount of delivered solution is directly affected by its property, leading to a situation where the actually delivered amount will change not only when the solution used or its concentration is changed, but also when the temperature of the solution or ambient environment alters.

We have developed a new method where first a desired accurate amount of solution to be delivered is fed and stored in the divided injection head by pushing the piston in a syringe, and then that amount of solution is delivered by jetting air. This method allows the injection of a specific amount of solution even if the solution or its property is changed, and also enables the accurate non-contact injection of a small amount of solution through the technique of spouting solution.

Figure 5 illustrates the basic structure of the divided jet injection dispenser. A signal is sent to the motor that drives the syringe piston, so that a desired amount of solution will be provided. The piston slides to feed that amount of solution into the injection head. Immediately after this action, the solenoid valve opens and the compressed air is supplied to the injection head to spout the solution.

The solution in the syringe is injected in divided amounts as

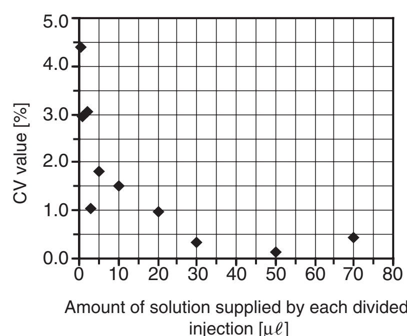

Figure 6 Results of Experiments Evaluating the Difference of Injection Amounts

described above. When the quantity of the solution in the syringe decreases, it can be supplied from the solution container by switching the three-way valve, allowing for continuous divided injection.

We have performed experiments using a 100 μℓ syringe and an injection needle of 0.3 mm internal diameter, with the amount of each divided injection ranging from 0.5 to 70 μℓ. From the experiments, it has been confirmed that the CV value for 20 injections falls with in ±5%. Figure 6 shows the results of the experiments. We have also conducted experiments using solutions with different viscosity ranging from 1.0 to 3.8 mPa·s (viscosity was changed by adding ethylene glycol to pure water). As a result, we have confirmed that the difference among each divided injection amount is within 1%, and each CV value (standard deviation/average value) falls within ±5%.

CONCLUSION

We reported in this paper some of the major elemental technologies used in the prototype of the cultured cell-based drug test system we have developed. Images obtained from this drug test system can be processed visually and mathematically based on the techniques described in other paper of this special issue. We will continuously work hard in the field of drug discovery and development by leveraging the resulting images as an effective tool to provide customers with solutions. ◆

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* ‘CSU’ is the registered trademark of Yokogawa Electric Corporation.