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### Publication Date

1991-05-01



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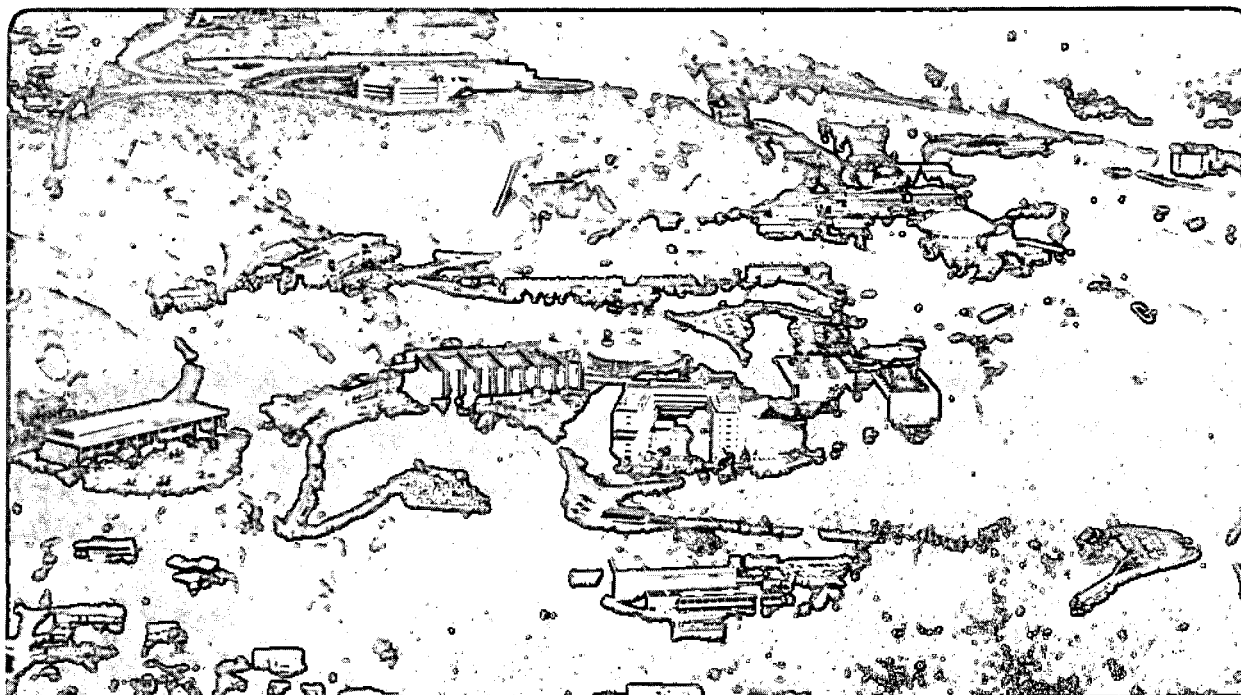
## Engineering Division

Submitted to Laboratory Robotics and Automation

### Application of Robotics and Automation in a Genomic Laboratory

J.M. Jaklevic, A.D.A. Hansen, E.H. Theil, and D.C. Uber

May 1991



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**Application of Robotics and Automation in a Genomic Laboratory**

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May 1991

This work was supported by the Director, Office of Energy Research,  
Office of Health and Environmental Research, U.S. Department of Energy  
under contract DE-AC03-76SF00098.

## **1 Abstract**

We describe an evolutionary approach to the development of automation for application in genomic laboratories. The first phase of the approach employs general purpose robots in prototype situations to investigate the potential for automation of a particular task. This work identifies the needs for mechanization, for development of special purpose tools and manipulators, and for information and database hardware and software support. It also identifies the bottlenecks in the overall process leading from experiment concept through the large-scale manipulation of samples to the final result. Based on this experience, the second phase may then proceed with the development of specialized sidestations dedicated to the rapid performance of particular tasks. These units are designed to perform discrete functions, but can be readily adapted for operation within a laboratory robotic environment. We illustrate this approach with examples of clone library replication and colony picking. The overall emphasis is on flexibility and upward compatibility, so that automation engineers may respond to the evolving needs of biologists. The processing power conferred by the new systems may itself enable the performance of experiments not previously feasible.

## **2 Introduction**

Among the goals of the Human Genome Initiative are the construction of various types of maps of individual human chromosomes and the sequencing of significant portions of genomic DNA. These efforts may be extended to characterization of genomic DNA of other species. In addition to the scientific merit of such initiatives, the magnitude of these ventures has the potential to impact the techniques of molecular biology and genetics through the large scale implementation of laboratory automation. The quantity and variety of laboratory material required, the large number of repetitive biochemical procedures, and the volumes of data resulting from the project challenges biologists and engineers alike to devise new methods for dealing with the greatly increased scale of operations.

These points can be illustrated by considering current activities in genomic mapping. Existing approaches to mapping involve the creation of large clone libraries of a size not seen heretofore. For example, a representative human genomic yeast artificial chromosome library [1] consists of 50,000 – 60,000 clones. The first few of these libraries are now in distribution and will provide a rich source of laboratory material for experimentation for years to come. The sheer amount of data and material handling for any one copy of the library is significant, as is the associated bookkeeping and sample tracking that must form an integral part of the experiments. If one looks forward to a time when the use of such libraries becomes a part of pharmaceutical research on a large scale, the need for accurate data and detailed record keeping may become a requirement for formal quality assurance.

Approaches to the problems of laboratory automation need to recognize the differences between a research laboratory oriented toward large scale experiments and a production environment in which specific tasks are designed for repetition over a long period of operation. We have adopted an evolutionary "bottom up" approach in which individual tasks are being addressed with the knowledge that each forms only a piece

of a larger picture, but also that the whole picture is not yet fully understood. Equipment to perform different tasks must be modular, with the capability of being linked at some future time. This is more difficult to do in hardware than with software but is still an appropriate approach if one can identify clearly generic tasks. By contrast, a "top down" design, as may be appropriate in a production situation, implies that all the requirements for an overall system design are understood. In a research environment with rapidly changing technology, that is not the case.

Our initial automation projects are directed at developing methods for manipulation of elements in the large clone libraries which are currently being used in DNA mapping studies by LBL biologists. These include colony picking and arraying, large scale library replication, and methods for generation of high-density hybridization arrays on membrane substrates. Our preliminary efforts have employed a commercial, programmable robot to manipulate 96 well microtiter plate arrays. Experience with this approach has indicated limitations in our particular applications. Second generation projects are currently underway in which some of the limitations of existing hardware are being overcome with the use of dedicated sidestations designed and built in our laboratory.

### **3 Existing Applications**

#### ***3.1 Plate Replication***

A primary resource for mapping studies is an arrayed library of cloned fragments of a larger DNA segment such as a complete chromosome. Such libraries are being generated by a number of laboratories both for use in their own research and to be shared as a resource with others. Current libraries may consist of as many as 50,000 to 60,000 unique fragments of human DNA, each growing in a colony of yeast or bacterial cells in liquid medium in one well of a microtiter plate. These 8.5 cm x 12.5 cm x 1.5 cm plastic plates, commonly used in microbiological and pharmaceutical research, contain an 8 by 12 grid of 250  $\mu$ L round wells. A library thus consists of 500 to 600 such plates. Use of the same library by different laboratories requires that efficient, cost-effective replication of these plates can be reliably performed.

The replication task illustrates how a general purpose robot may be customized to perform specific jobs. The Hewlett-Packard Microassay System we are using (Fig. 1) consists of a computer-controlled arm with a cylindrical coordinate system, stackers for microtiter plates and pipet tip racks, a gripper hand for manipulating plates, tips, and other labware, and eight programmable syringes for pipetting and dispensing fluids into up to sixteen microtiter wells at a time. The software supplied by the manufacturer includes an interpretive language similar to BASIC, and the facility to teach primitive motions manually.

To this we have added a custom-built replication tool containing 96 stainless steel pins (Fig. 2), with which the robot can transfer cells from one microtiter plate to another in a single motion. The robot manipulates this tool with its gripper hand. To prevent cross-contamination between plates, the tool is sterilized between use by sonication in an

ethanol bath followed by heating. The robot saves time by fetching and storing plates during the sterilization periods.

Using this specialized hardware, it takes 3.1 hours of unattended operation to make four copies each of twenty source plates, including dispensing fresh growth medium and glycerol (to preserve the cells when frozen) into the copy plates. At this rate, four copies of a 600 plate library could be made by one person in twenty working days.

### **3.2 Colony Picking**

In another application area, we have combined image processing and robotics to automate the task of colony picking and arraying [2]. This job consists of picking up cells from small yeast or bacteria colonies scattered at random over the surface of agar in Petri dishes, and transferring them into growth medium in wells of microtiter plates, one colony per well. The purpose is to put the DNA library growing in these colonies into an ordered format so that it may be readily replicated and otherwise processed. The overall system is shown conceptually in Fig. 3.

Our initial approach once again was to adapt a general purpose robot with specialized hardware. We first obtain a digital image of each Petri dish with a camera and digitizer. The 100 mm x 100 mm square Petri dish is manually placed in a holder of our design (Fig. 4) which registers the dish in place on a light table under the camera. The holder contains three fiducial reference marks which determine a local rectangular coordinate system for the dish. Using commercial software, the operator acquires an image and its fiducial points, and interactively establishes a rectangular area of interest and a grey-level detection threshold which clearly identifies as many colonies as possible while minimizing the overlap of adjacent colonies. The image-processing software computes the centroid coordinates of the colonies and writes these values to a floppy disk file.

Since the imaging process currently requires considerable user interaction, it is done separately from the picking operation, which is fully automated on the Hewlett-Packard robot. We have equipped the robot with a Petri dish holder (Fig. 5) identical to the one on the imaging station, as well as with custom stackers for the dishes, and have taught it the necessary motions to fetch a dish from the stacker, place it in the holder, remove and replace the lid, and return the dish to another stacker. Disposable pipet tips are used as the actual picking implements, and we have built a special tool with a long shaft which allows the robot to hold the tips straight to ensure accuracy (Fig. 5).

The robot can process up to ten Petri dishes per run. At the start of the run, the user loads the dishes in a stacker in the same order and orientation as used during imaging, and enters the names of the floppy disk files containing the colony coordinates. As the robot moves each dish from the stacker to the holder, it reads the appropriate file. The control program computes the robot coordinates of each colony, based on its location relative to the three reference marks, and transfers cells from the colony to a microtiter plate well using a new pipet tip for each colony. The robot loads new dishes, plates, and tips from their stackers as needed. The system has operated unattended in overnight runs up to twelve hours.

#### **4 Limitations of Present Methods**

Our experience with these applications has given us several insights about adapting general purpose equipment to new applications. First, the system must have an inherently open architecture to permit new hardware and software to be added readily. The Hewlett-Packard robot has served well in this regard. Second, a general purpose system is a good vehicle to test a new concept, such as colony picking, to determine the critical areas for accuracy and speed, but it will in general not be optimal. The Hewlett-Packard system was designed to work with microtiter wells 6 mm in diameter, and is barely capable of the 0.5 mm accuracy needed to pick small colonies. It can pick only 150 colonies per hour, the slowness largely being due to the need to obtain a new pipet tip for each colony. While the robot can operate tirelessly for hours, an experienced human can pick colonies at four times this rate.

We also recognize that the imaging process has several limitations. For example, we manually select the thresholds and areas of interest for each Petri dish. Better control of lighting conditions and camera position would allow the image processing software to make these selections automatically. In addition, we currently pick all objects except those with large enough area to be obvious artifacts. However, our software package gives us the ability to make various size and shape measurements, so that we could reject overlapping colonies based on roundness or aspect ratio, or even segregate colonies into classes of different size or shape.

Another limitation of the current system is the need to manually teach the robot the depth of the agar in each batch of Petri dishes, so that each colony is penetrated by a pipet tip, but not so deeply that nearby colonies are displaced. Variations in depth can be caused by pouring the agar nonuniformly, or by subsequent shrinkage.

#### **5 Dedicated Sidestation for Automatic Colony Picking**

In order to overcome some of the current limitations, we have started work on a special purpose device dedicated to the task of rapid, automatic colony picking. The goal is to develop a unit that will transfer colonies from random locations on an agar surface in a source dish to an ordered matrix of destination wells in a microtiter plate, at a rate considerably greater than that presently achieved by the general-purpose robot arm. Our target was to improve the speed by at least one, preferably two orders of magnitude over the existing system. The initial goal is to pick and place colonies at a rate of one per second.

Conceptually, the dedicated colony picker consists of four major units as follows: 1) a platen of controllable X-Y movement on which the source dish of randomly-located colonies is placed; 2) a similar platen on which the destination microtiter plate is placed; 3) a carousel wheel fitted with a large number of reusable plunger needles around the circumference, rotating above the two dishes; and 4) a sterilization zone in which the needles are cleaned before re-use. Figures 6 and 7 illustrate the design. The primary function of the unit will be to transfer material from locations  $(x_i, y_i)$  of the randomly located colonies on the source dish, to locations  $(x_j, y_j)$  on the grid of the destination microtiter plate. The unit is intended to be serviced by the general-purpose



robot in terms of being loaded and unloaded with full and empty plates. If a picking rate of one colony per second is achieved, then destination microtiter plates will need to be unloaded and reloaded every 96 seconds. Source colony plates, containing possibly hundreds of colonies, will have to be changed every few minutes.

The sidestation operates as follows: The source colony dish will be loaded onto one platen, and locked into place so that its position is known relative to the intrinsic X-Y coordinates of the platen. Similarly, a destination plate will be placed on the other platen. The file of X-Y coordinates of the colonies will be loaded into the computer operating the system, and the system will start to run. First, the colony plate is moved by the X-Y table so that a colony is directly underneath the needle picking position. Simultaneously, the carousel is rotated so that a needle is positioned above the plate and underneath a plunger. When the "pick" positions are verified by encoders, the plunger will be actuated and the needle dipped into the colony and then retracted. The carousel will then rotate so that the needle carrying the colony cells is directly above the correct well of the destination plate, which has also been properly positioned. When the "place" positions have been verified, a second plunger will dip the needle into the medium in the destination plate so that some of the cells are transferred. The carousel will then rotate the needle through a sterilization zone (sonicating liquids, heat, etc.) so that when it returns after 360° it is ready to pick the next colony.

The above outline describes only one picking needle, and is limited in throughput by the time necessary for sterilization and the time required to move the carousel wheel through the various rotations. We are planning to employ many needles around the circumference of the carousel wheel, so that at any instant one is picking cells from the colony plate, one or more are in transit, one is placing cells in the destination microtiter plate, and all of the rest are being sterilized or cooled off ready for re-use. Our present design can accommodate 24 needles spaced at 15° intervals around the circumference. Combining this with the manufacturer's specifications of speed and accuracy for the X-Y table motions, we believe that one pick per second should be easily attainable.

The coordinate locations of the colonies will need to be passed to the system in this configuration. We are designing the system so that a later extension could add machine vision to the colony plate platen. The X-Y table for the source plate has an open center, so that the plate can be placed on a transparent lucite surface and illuminated from below. A camera mounted above would then be able to image the plate and pass the video frame to a digital analyzer. The image analyzer should be able in principle to locate all of the colonies, and differentiate "good" colonies from bubbles in the gel, miscellaneous intrusions, etc., based on the criteria of size and shape as discussed in the previous section. The coordinates of the "good" colonies would then be expressed in terms of their X and Y positions on the platen. Since the colony plate is locked onto the platen and not moved between imaging and picking, it will not be necessary to re-locate it against fiducials. A picking cycle might thus progress as follows: 1) colony plate is locked onto the source platen; 2) moved to "imaging" position; 3) image analysis identifies and locates colonies; 4) colony plate is moved to "picking" area; 5) smaller, rapid movements position the colony plate under the needle picking position as successive stitching movements pick cells from each colony, and transfer them to the destination plate(s); 6) the completely-picked plate is moved out to the

edge of the working area, unloaded, and a new plate is loaded for the cycles to continue.

The proposed sidestation will consist of a pair of X-Y stages with associated software control and an on-line camera and image processing system operating in the same computer. Operation of the general purpose robot will be integrated with this system in order to provide a versatile platform with which to efficiently address a number of laboratory automation tasks. Immediate applications besides high-speed colony picking could include preparation of high-density hybridization arrays, high-speed fluorescent scanning of 96 well ligation assays, and implementation of a variety of colony pooling approaches.

## 6 Discussion

We have described an approach to laboratory automation which is still evolving but in which the main elements are becoming clear. It uses general purpose robots, both as prototyping tools and as loaders and feeders for specialized sidestations. The sidestations are designed to be independent of any particular feeder mechanism, so they may be linked to different general purpose robots. As prototypes, the robots are very useful in helping to identify bottlenecks and the need for ancillary equipment (e.g., special holding devices or sterilizers). At the same time, the approach does not rule out their use as the preferred device when that is appropriate, as in the case of library replication. An important task now is to integrate these devices with others that will prove useful in a genomics laboratory. Integration will require more than communication links between machines; a brief description of elements already identified includes the following:

- Bar code readers integrated with the robots. Bar codes of laboratory material will facilitate the use of a database. This would permit, for example, the ability to easily retrieve clones which have been shown to have interesting characteristics as a result of probe hybridizations or other tests.
- Integration of image acquisition and analysis with hybridization experiments. The screening and characterization of clones against a panel of known probes is one of the principal analytical tools in the genomics laboratory. This is done frequently, and so it is critical that it be as simple and reliable as possible. This implies a database of hybridization results, including precise filter and plate locations of individual clones.
- A high level robotics language. It is important that the existing software bottleneck be circumvented by a more graphic, user-oriented approach. The result will provide a system that is easier to use, does not require frequent expert computer support, and electronically captures protocols and parameters automatically.

While this short list could obviously be extended, it serves to illustrate the kind of biological research tasks that lend themselves to automation. However, in a research laboratory the impact of automation may be more subtle than in an assembly line, where it is usually seen as a way to reduce tedium and make processes more cost-

effective. While those are valuable goals, the greatest value of automation in biology research may lie in the power it gives to researchers. For example, experimental techniques such as sequencing via hybridization are only practical with automatic methods and data capture. As another illustration, the use of a high level language for controlling robots is more than just a convenience; it provides a tool with which a researcher can alter protocols and parameters simply, thereby permitting the exploration of many variations of existing ideas or new methods. At the same time, the specification of these changes are automatically captured in the language and available for electronic log keeping and analysis.

In this way, robotics and automation can provide new research tools beyond merely the alleviation of tedium. One of our goals is to contribute to this capability for the genome community.

## **7 References**

1. B.H. Brownstein, G.A. Silverman, R.D. Little, D.T. Burke, S.J. Korsmeyer, D. Schlessinger and M.V. Olson, "Isolation of Single-Copy Human Genes from a Library of Yeast Artificial Chromosome Clones", *Science* 244, 1348-51 (1989).
2. D.C. Uber, J.M. Jaklevic, E.H. Theil, A. Lishanskaya and M.R. McNeely, "Application of Robotics and Image Processing to Automated Colony Picking and Arraying", *Biotechniques*, in press.

## **8 Acknowledgment**

This work was supported by the Director, Office of Energy Research, Office of Health and Environmental Research, U.S. Department of Energy under contract number DE-AC03-76SF00098. Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

## 9 Figure Captions

Figure 1: The Hewlett-Packard Microassay System. The robot arm with attached gripper is at left background.

Figure 2: Robot replicating a human DNA library. The robot is lowering a custom-built 96-pin tool into the wells of a 8.5 cm x 12.5 cm microtiter plate. Yeast cells will cling to the stainless steel pins and be transferred to identical replicate plates.

Figure 3: Basic elements of the colony picking system. An imaging system locates colonies of cells containing human DNA fragments, growing in Petri dishes. A robot then transfers cells from each colony into a well of a microtiter plate.

Figure 4: A camera imaging the colonies in a Petri dish. The 100 mm x 100 mm dish is held in a custom fixture on a light table. The image is captured by a frame grabber on a PC, which locates the colonies with image processing software.

Figure 5: Robot picking a colony in a Petri dish using a sterile pipet tip. The disposable tip is held straight by the long shaft inserted into it. The robot has placed the dish in a custom fixture which keeps the dish in place with the spring-loaded clip in the foreground.

Figure 6: Design for dedicated sidestation for colony picking, top view. The colony source plate is placed on the left XY platen and the destination microtiter plate on the right platen. Picking needles around the circumference of the carousel are dipped into the colonies on the source plate, and then rotated to place the colonies in the wells of the microtiter plate. The needles are sterilized before returning for re-use.

Figure 7: Design for dedicated sidestation for colony picking, side view (see caption for Fig. 6). The colony source plate and the destination microtiter plate are loaded and unloaded by a general purpose laboratory robot.



FIGURE 1

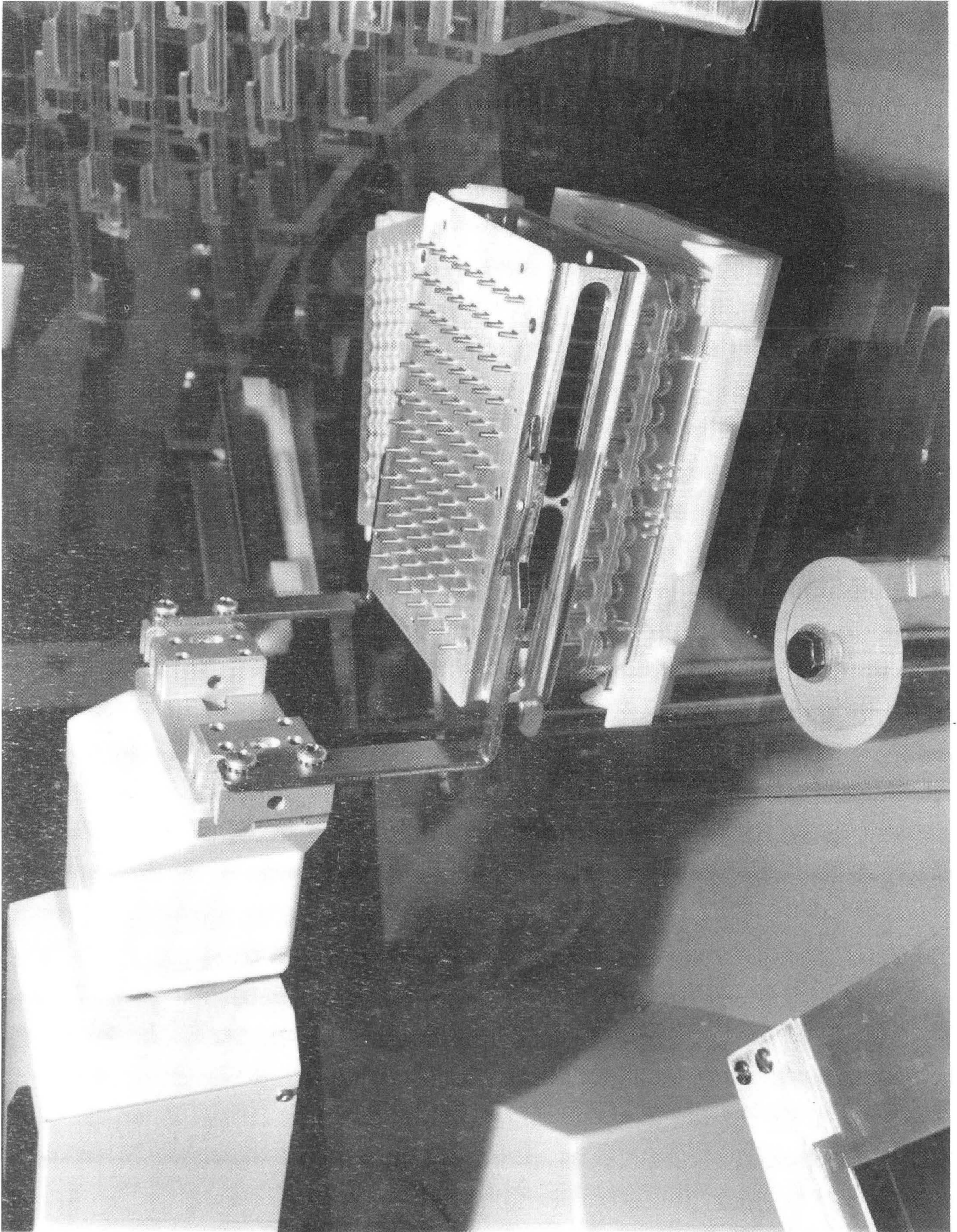


FIGURE 2

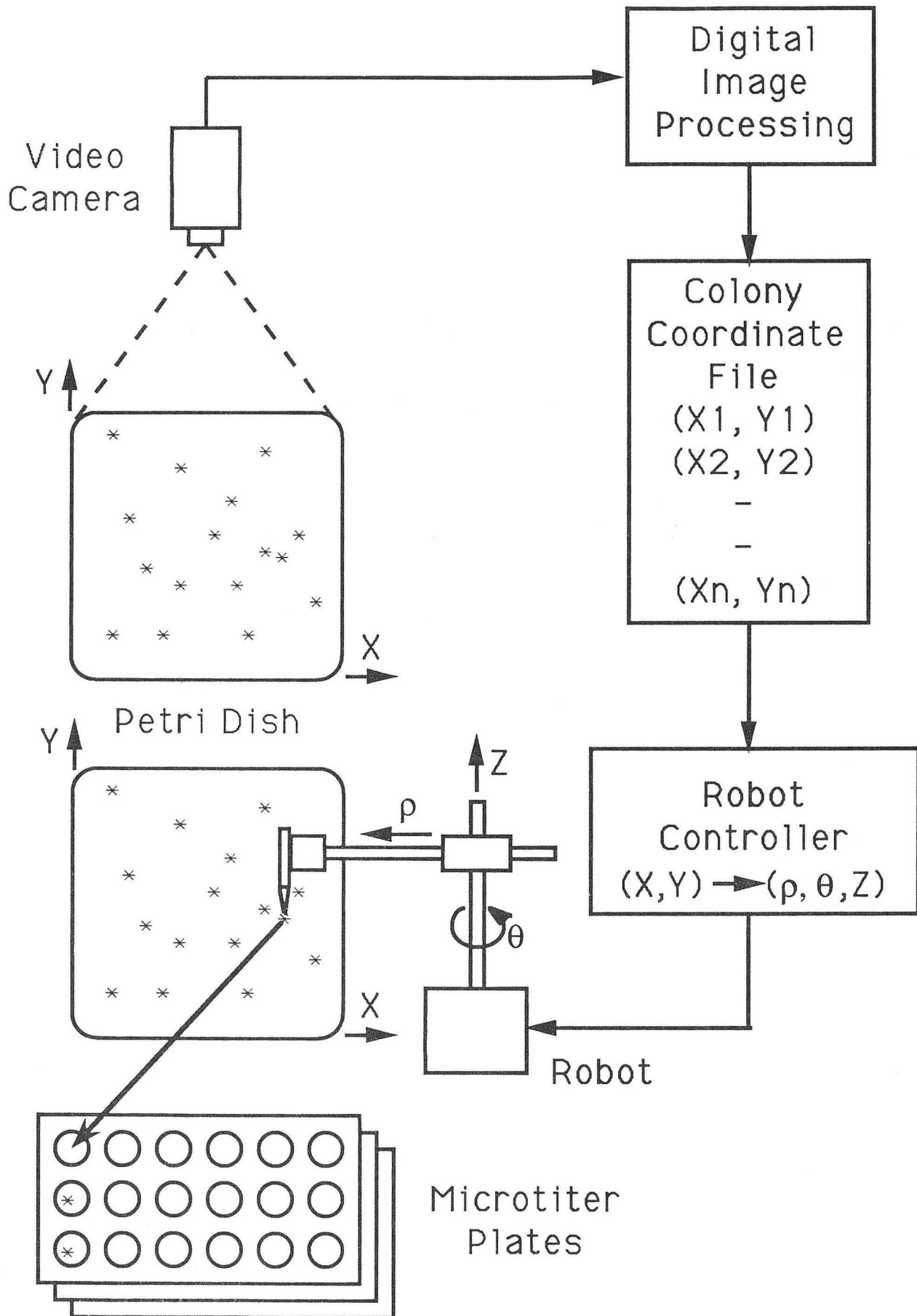


FIGURE 3

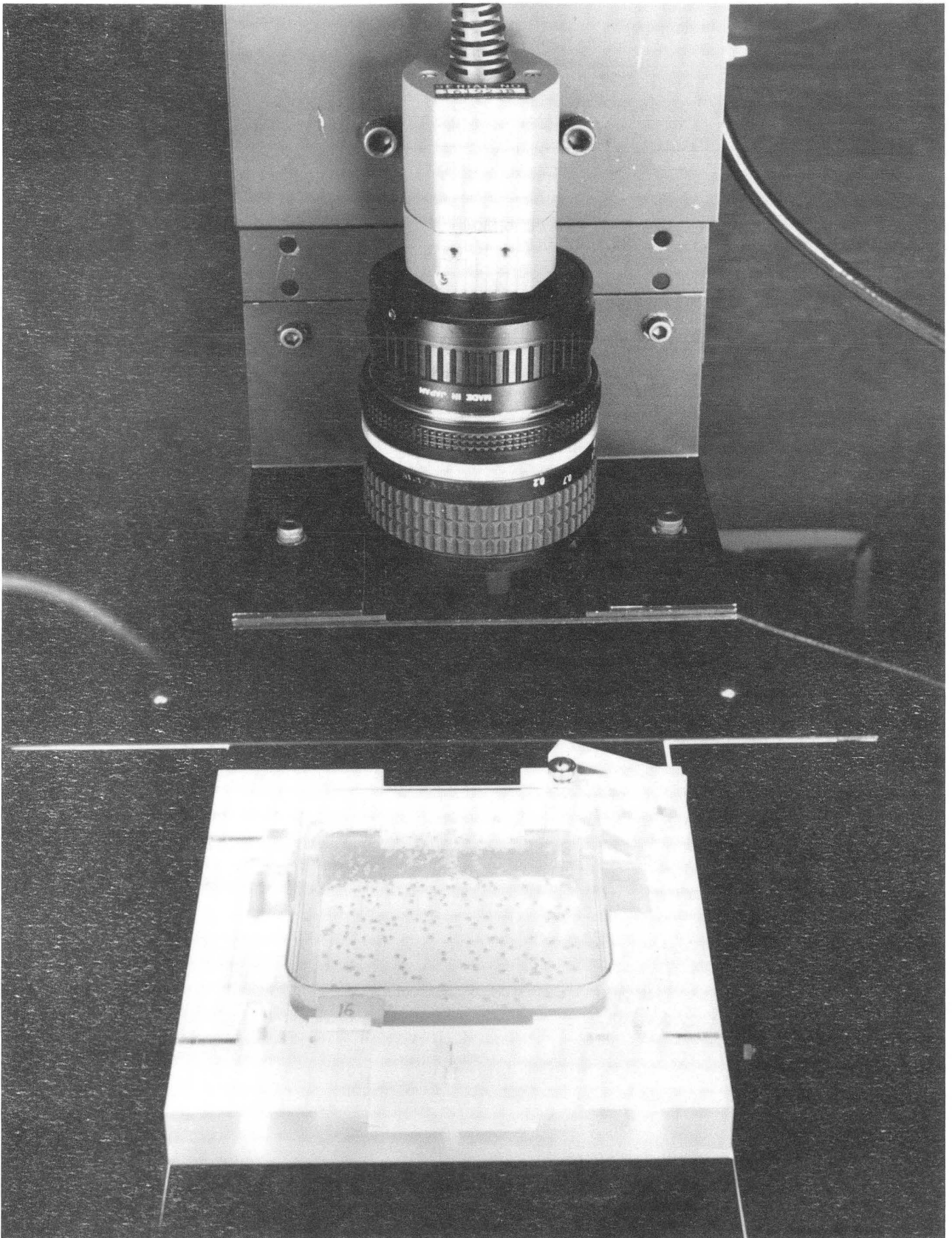


FIGURE 4



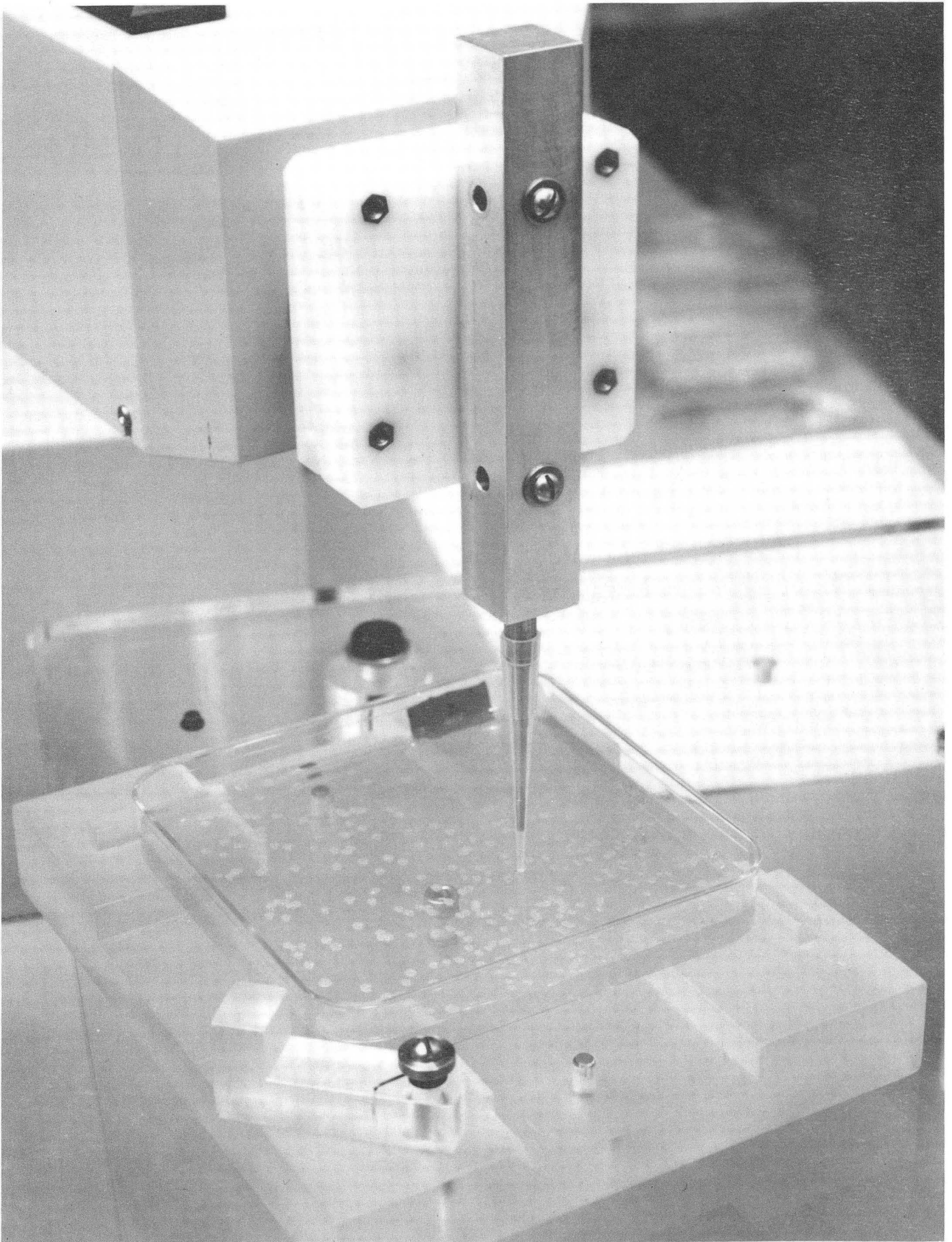
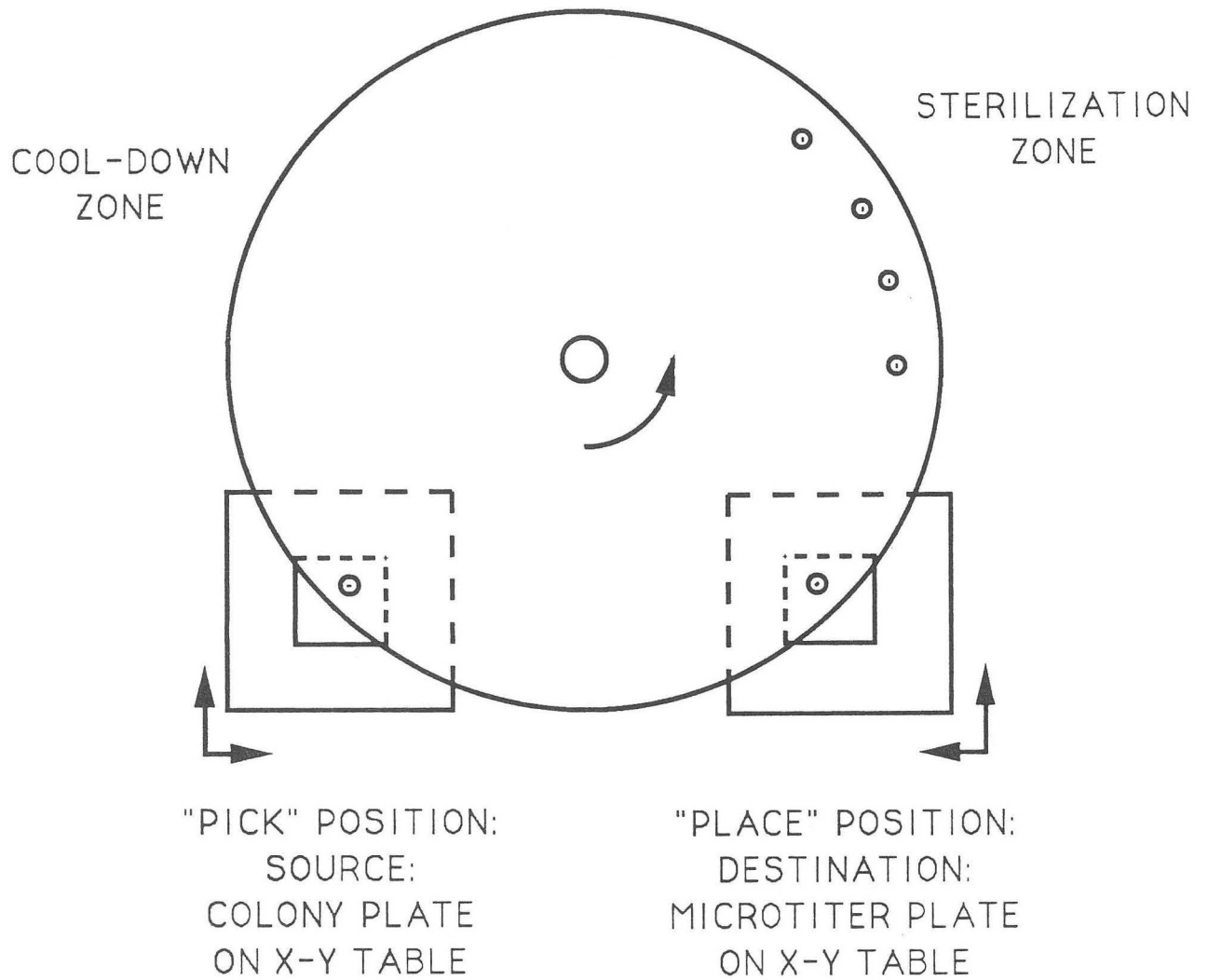


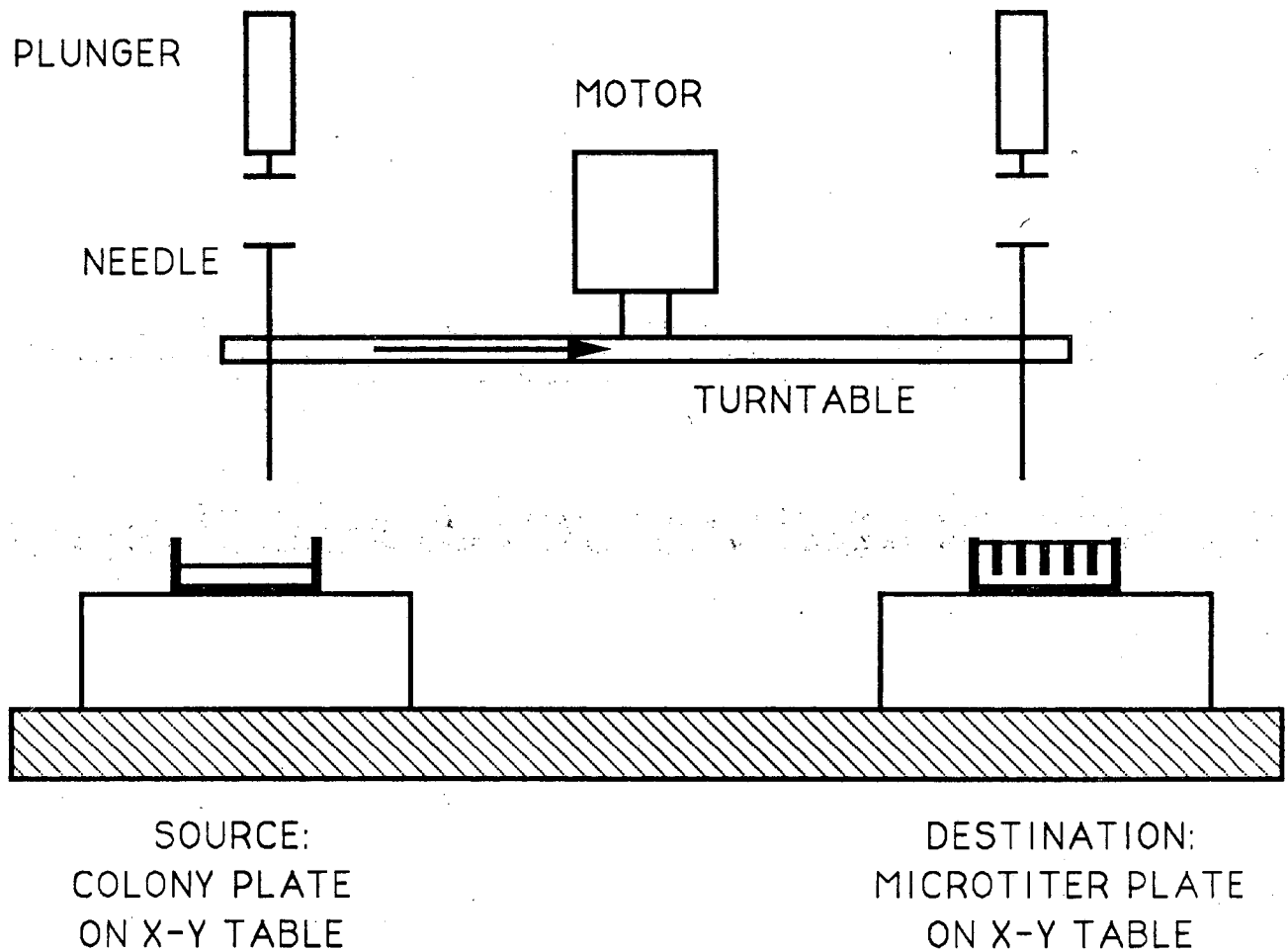
FIGURE 5



## COLONY PICKER: CONCEPTUAL DESIGN

### TOP VIEW

FIGURE 6



## COLONY PICKER: CONCEPTUAL DESIGN

### SIDE VIEW

FIGURE 7

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