

Introduction to Microinjection

Single-cell microinjection is a powerful and versatile technique for introducing exogenous material into cells and for extracting and transferring cellular components between cells. Any microinjection system will typically comprise three basic functions: the imaging of the target cell and the micropipette, the positioning of the micropipette, and control of the pressure inside the micropipette. The **XenoWorks™ Microinjection system** has been devised to fulfill the requirements for the last two elements and to be versatile enough to be used for most microinjection and micromanipulation applications on a number of different microscope platforms.

General Considerations

Workstation

Effective, reproducible microinjection requires a suitable quiet location, away from sources of vibration (slamming doors, elevators, centrifuges etc.), fluctuating temperature, drafts and bright light. In addition, convenient access to facilities such as incubators, compressed air (for antivibration tables) and operating theaters needs to be considered where necessary. Limiting the amount of vibration to the microinjection workstation is vital. The measures which need to be taken will depend upon the amount of ambient vibration. In general, the workstation should be set up in a quiet room, with little or no foot traffic, no slamming doors or heavy machinery such as elevators, centrifuges and refrigerators. Select a heavy, sturdy table or bench with plenty of room surrounding it, and ensure that the workstation components, including trailing wires and tubes, are not in contact with the floor or walls. If vibration is still detected (this can be judged by projecting a micropipette into the microscope field of view under high magnification and noting the behavior of the micropipette tip), use of an antivibration table should be considered. These can be home-made from a solid table with a thick metal or marble slab placed on top of bags of sand or small semi-inflated tire inner tubes. Commercially available air tables are extremely effective in reducing vibration over a wide range of frequencies.

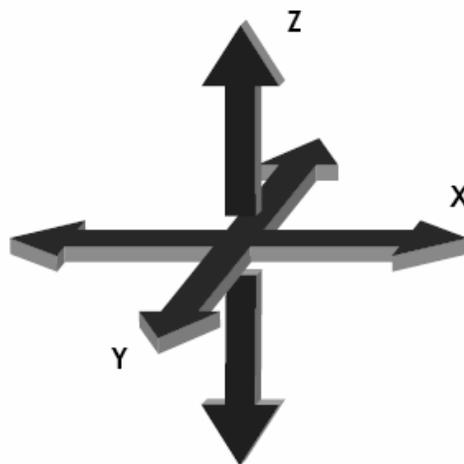
Microscope

An *inverted microscope* (one whose objective lens turret is located below a fixed stage, with the transmitted light source located above) is typically used for microinjection applications. Of particular importance is the *working distance* of the condenser, which is measured from the bottom-most part of the condenser to the stage, after the condenser has been adjusted correctly. Ideally, a “long” or “ultralong” working distance condenser should be used to permit the micromanipulator headstages access and unobstructed movement. Similarly, the use of stage attachments such as moving stages, specimen holders and heating/warming inserts should be considered as possible sources of conflict with micromanipulator motors. The addition of a camera and TV monitor should be considered and their inclusion is essential if the system is to be used for teaching or demonstration purposes (note, however, that not all microscopes are supplied with appropriate camera ports).

Micromanipulators

The micropositioning device – the micromanipulator – should be securely mounted to the microscope by means of an appropriate adapter (supplied separately). The performance of the micromanipulator and ultimately, the success of any microinjection will depend in large part upon the stability of the micromanipulator mounting. The most stable micromanipulators are mounted on as large a footprint as possible, with little overhanging mass between the microscope mounting and the position of the pipette holder clamp. The micromanipulator is used to position and move the micropipette in proximity to the tissue to be manipulated or injected. In electronic micromanipulators such as the **XenoWorks micromanipulator**, the joystick position is sensed

and its position is interpreted by software which in turn drives three motors set at right angles to each other, representing the three major geometric axes, X, Y and Z (see figure below). The advantages of electronic motor micromanipulators are: (1) the software is “aware” of the micropipette position and so can be programmed to move to any pre-determined spatial coordinate within the system’s range, and (2) fine movement can be preserved across the entire travel of each motor (hydraulic and mechanical systems, in contrast, have a travel range limited to a few millimeters and require an additional coarse positioning system).



Microinjector

The microinjector is used to control the pressure level in the micropipette. Depending upon the application being employed, different pressures may be required. These applications are described below. Direct-pressure microinjection of fluids such as nucleic acid solutions, dyes and drug compounds into cells uses a sharp hollow capillary-glass micropipette with a tip diameter typically less than one micron. Considerable pressure (in the order of a few hundred hPa) is required to force the solution out of the tip of such a micropipette and into the cell. While injection pressure is not being applied, a constant positive “base” pressure should be applied to the tip to ensure that natural capillary action does not draw the medium in the injection chamber, up into the micropipette, and dilute the solution being injected. In the **XenoWorks digital microinjector**, injection and base pressures are independently controllable. The injection system must be capable of delivering a pulse of high pressure to the micropipette tip to clear out the blockages that inevitably occur when injecting through the lipid cell membrane. In addition to variable pressure, the injection system is able to be programmed to deliver pulses of pressure for defined times, usually in the order of tens of milliseconds, on command. In this way, fairly reproducible injections are possible from one cell to the next (see also “Quantitative Microinjection of Solutions” below) simply by pressing a single switch. Finally, the desired pressure may be delivered continuously as long as a control (such as a footswitch) is depressed.

Quantitative Microinjection of Solutions

The direct determination of the quantity of solution delivered to an individual cell is a complex procedure, but in many cases unnecessary. Most cells cannot tolerate being injected with more than about 5% of their starting volume, and this must be taken into account when designing experiments. More information on quantitative microinjection is available from references [1] and [2].

Cellular manipulation and reconstruction

The aspiration, transfer and insertion of cellular components is performed with micropipettes with much wider tip diameters than those used for direct pressure microinjection. A considerably narrower spectrum of positive and negative pressures is required, and a greater level of control must be exercised over the pressures applied. Traditionally, control of these narrow pressure ranges has been achieved with the use of a micropipette holder connected by narrow-gauge tubing to a precision syringe whose plunger is connected to a micrometer lead-screw. By turning the screw, and thus varying the volume of the syringe, very precise pressure control in the micropipette is achieved. By using an hydraulic fluid instead of air in the syringe and tubing, an even greater fidelity of control is attained. The **XenoWorks analog microinjector** is just such a device and can be used for many applications (see Section 8.3 below for details). Several factors should be considered when optimizing the performance of such hydraulic microinjection instruments:

- Hydraulic tubing should be kept as short as possible to ensure good transmission of hydraulic pressure from syringe to micropipette tip.
- Some hydraulic fluids give better performance than others under different conditions – Sutter recommends mineral oil such as that supplied by Sigma for tissue culture (0.84g/ml) although Fluorinert™, water and even air alone can give good results.
- Performance can be adversely affected if there are air bubbles in the hydraulic line connecting the syringe with the micropipette.
- Performance is also affected by the position of the air/oil interface at the back (blunt) end of the micropipette, and the culture medium/air interface at the front (sharp) end of the micropipette (see microinjector instruction manual operating instructions and troubleshooting sections for details).

The **XenoWorks digital microinjector** has also been designed to fulfill the narrow-spectrum pressure ranges required for embryo holding and embryonic stem cell transfer, and because it is also capable of providing high pressure for solution injection, it is an ideal instrument for a transgenic animal production workstation (see the XenoWorks digital microinjector manual for details).

Training

Most microinjection techniques can take many weeks, even months to grasp, and years to perfect. If microinjection is to become a regularly-used technique, consider attending a training course. Contact Sutter for details on training courses.

Microinjection Applications

The configuration of any microinjection system will depend upon the application for which it is used. Listed below are some of the more commonly-used applications:

Zygote pronuclear DNA microinjection

The microinjection of DNA into the pronucleus of a newly-fertilized mammalian egg is now a common and highly efficient method of creating transgenic offspring. Pronuclear microinjection was first described in the mouse [3], but now many different transgenic animals have been created in this way. Because the micropipette used for injection is sharp (the internal diameter at the opening typically less than one micron) relatively high pressure (>3000 hPa) is required to inject the DNA solution. Two micromanipulators are required, one to hold the zygote and one to inject the DNA. Gentle negative pressure is used on the holding side, while pulses of high pressure are used to inject 1–2 picoliters of DNA solution into the pronucleus. The XenoWorks digital microinjector is ideal for this application, having simultaneous holding and high-pressure injecting capabilities.

Suggested system configuration:

- 1 x XenoWorks Micromanipulator (Right) BRMR
- 1 x XenoWorks Micromanipulator (Left) BRML
- 1 x XenoWorks Digital microinjector BRE110/BRE220
- 2 x XenoWorks Microscope adapter BR-xxx

Embryonic stem cell transfer into blastocyst

Animals, usually mice, can be engineered with a specific gene function reduced or knocked out altogether by introducing genetically altered embryonic stem cells into the cavity of a blastocyst so that the stem cells contribute to the embryo [4]. The resulting live animal is a chimera of both genotypes. Subsequent selective interbreeding of manipulated animals results in pure-bred gene “knock-outs” or “knock-downs” and can be used for subsequent gene function studies. The introduction of embryonic stem cells into a blastocyst requires two micromanipulators, one for holding the blastocyst and one for transferring the cells. Both holding and transfer functions require gentle positive and negative pressure for which the digital microinjector is ideal. Alternatively, two analog microinjectors may be substituted.

Suggested system configuration:

- 1 x XenoWorks Micromanipulator (Right) BRMR
- 1 x XenoWorks Micromanipulator (Left) BRML
- 1 x XenoWorks Digital microinjector BRE110/BRE220 OR:
- 2 x XenoWorks Analog microinjector BRI
- 2 x XenoWorks Microscope adapter BR-xxx

Somatic cell nuclear transfer

The enucleation of an oocyte followed by the transplantation of a somatic cell [5,6] is a method of producing genetically identical copies (clones) of the animal from which the donor cell was taken. Generally, two micromanipulators are required, one for holding the oocyte and one for the enucleating and injecting procedures. Each micromanipulator grips a single micropipette holder with a microinjector attached. Oocyte holding, enucleation and somatic cell transplantation require gentle, low positive and negative pressure, making the analog microinjectors ideal.

Suggested system configuration:

- 1 x XenoWorks Micromanipulator (Right) BRMR
- 1 x XenoWorks Micromanipulator (Left) BRML
- 2 x XenoWorks Analog microinjector BRI
- 2 x XenoWorks Microscope adapter BR-xxx

Intracytoplasmic Sperm Injection

Intracytoplasmic sperm injection (ICSI) can be employed for veterinary in-vitro fertilization during rare species preservation or for any veterinary assisted conception [7]. ICSI may also be used as a gene transfer technique when sperm are co-injected with exogenous DNA [8] (see also Piezo-assisted microinjection, below). Typically, two micromanipulators are used, one for oocyte holding and one for sperm aspiration and injection. Each micromanipulator grips a single micropipette holder with a microinjector attached. Since gentle positive and negative pressures are required for the delicate task of oocyte holding and sperm injection, an ICSI workstation should be configured with two analog microinjectors.

Suggested system configuration:

- 1 x XenoWorks Micromanipulator (Right) BRMR
- 1 x XenoWorks Micromanipulator (Left) BRML
- 2 x XenoWorks Analog microinjector BRI
- 2 x XenoWorks Microscope adapter BR-xxx

Piezo-assisted ICSI

This relatively new technique can be employed for assisted conception in animals for whom standard ICSI fails, such as mice [9]. Piezo-assisted microinjection has been employed as a gene transfer method, where sperm are coated in exogenous DNA and injected into oocytes [8]. The microinjection workstation required for this technique is very similar to standard ICSI, but with the addition of a piezo impact drive attached to the injecting micropipette holder. The device vibrates the injecting micropipette axially and drills its way into the oocyte. This method has been shown to increase success rates. Because the micropipette is vibrating at minute amplitude but high frequency, it is vital to use a mechanically stable micromanipulator, which will not vibrate in sympathy. The more stable the micromanipulator, the more efficient the energy transfer from the piezo impact drive to the micropipette tip. Most piezo-assisted microinjection protocols currently require a bead of mercury to be used to stabilize the injecting micropipette. Mercury should not be used in conjunction with the digital microinjector, though the analog microinjector is ideal for this purpose.

Suggested system configuration:

- 1 x XenoWorks Micromanipulator (Right) BRMR
- 1 x XenoWorks Micromanipulator (Left) BRML
- 2 x XenoWorks Analog microinjector BRI
- 1 x PMM-150FU piezo impact drive with XenoWorks adapter
- 2 x XenoWorks Microscope adapter BR-xxx

Microinjection of cultured, adherent cells

Cultured cell lines such as 3T3, CHO and HeLa can be microinjected while attached to a Petri dish [10, 11]. The procedure is best viewed through phase-contrast optics; a single micromanipulator and a single high-pressure microinjection channel are required. The tip of a sharp (inner diameter less than 1 micron) micropipette is brought down on top of a single cell and a pulse of high (100–1000 hPa) pressure applied. The cell membrane is ruptured and the cell can be seen to inflate slightly. Typical volumes injected are typically less than 5% of the cell volume. Success rates vary widely depending upon the type and volume of compound injected, the culture conditions and the cell line used. The high-pressure function of the digital microinjector and the smooth, fine control of the XenoWorks micromanipulator are particularly well-suited to this application.

Suggested system configuration:

- 1 x XenoWorks Micromanipulator (Right) BRMR
- 1 x XenoWorks Digital microinjector BRE110/BRE220
- 1 x XenoWorks Microscope adapter BR-xxx

Other microinjection applications

Please contact Sutter for assistance in configuring other microinjection systems.

Questions/Comments?

Call (415) 883 0128 or email robk@sutter.com.

References

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