History of the Custom-Modified Narishige Micromanipulator used in Mario Capecchi's seminal DNA-microinjection experiments and present in the display here

History of the Custom-Modified Micromanipulator used in Mario Capecchi's first DNA-microinjection 'gene-targeting' experiments.

This is a brief description of a quite trivial design modification I had incorporated in a popular micromanipulator widely used for cell biology and neurophysiology experiments involving penetration of cells with glass micropipettes or micro-electrodes. The modification, trivial though it was, provided an affordable instrument borrowed by Mario Capecchi, a friend and colleague in a neighboring Biology lab at the University of Utah, whom I had already known for several years from shared time in neighboring Biochemistry labs at the Harvard Medical School. Mario used the modified manipulator for early experiments that ultimately resulted in the Nobel-prize winning work described elsewhere in this binder.

In 1971, shortly after I'd arrived in Utah to work on experiments with cultured neurons, I wrote to the Nariishige Scientific Instrument Laboratory in Japan asking for a custom modification of one of their popular micromanipulators, the MO-15.

The original MO-15 (Addendum 1) provided relatively standard movements in the horizontal (XY) plane and movement in the vertical (Z) axis. It offered an additional, very fine movement along the X axis, controlled hydraulically with a remote hand control connected to the manipulator by a very thin, flexible line. That fine control permitted advance of a micropipette along its own axis without communication of vibrations during the manual control. This was an essential requirement, e.g. for penetration of cultured neurons for electrical recording without destructive vibration-induced injury of their membranes.

However, since the hydraulic fine motion was along the X axis, the entire manipulator had to be tilted to provide an angled axis along which the micropipette could be advanced to penetrate cells on the bottom of an open culture dish resting on an inverted microscope and viewed from below the dish. To move the micropipette from cell to cell in the dish during selection of those to be penetrated, it was also necessary to have the micropipette moveable in an XY plane parallel to the dish bottom, slightly above the cells. While the tilted manipulator's Y-axis control still permitted easy movement in the Y direction of that plane, movement in the plane's X direction required a very awkward combined use of the manipulator's X- and Z- axis controls, moving the micropipette in a series of jagged, triangular, sawtooth-like steps. Once a cell had been chosen and the micropipette's tip positioned near it by that awkward combination of manipulator controls, the tip could be smoothly advanced along its axis, toward the chosen cell, by the fine, hydraulic control of the original, now tilted, X axis.

What I requested, was that Narishige use components of the MO-15, plus one part from other manipulators in its line, to create a version of the MO-15 with the following features:

1. The X-axis controls, coarse and hydraulic fine, be detached from the YZ-axes and controls of the original version, i.e., from the 'base' of the original version.
2. The Z axis of the remaining base be set horizontally to become a new X axis. In combination with the original Y axis, that would provide movements in an XY plane that could be set parallel to the microscope stage, as desired.

3. The original X-axis controls be mounted at the end of the now-horizontal old Z axis (the new X axis) via an adjustable ball joint (available from other manipulator models), allowing those original X-axis controls to be tilted at various angles with respect to the new XY axes.

The modifications would provide the desired easy adjustment of a micropipette in an XY plane set parallel to the culture dish bottom (microscope stage), and also movement of the micropipette along its own axis via both the coarse and hydraulic fine controls of the manipulator's original, now tilted X axis.

The proposed new design was sent to Narishige as a diagram (Addendum 2).

Narishige provided the modified version, essentially as described, at a relatively modest cost of $390 plus a $26 shipping charge. I ordered two of them. Mario borrowed one and, as noted, employed it with historic effectiveness.

Entertainingly to me, once Mario's seminal paper citing successful use of the modified Narishige manipulator (Addendum 3) had received quite wide recognition, others must have written to Narishige requesting the same, modified version. It appeared for a while as a standard offering in their catalog (Addendum 4) -- albeit at a price three times what I had paid for the custom original and that soon became nearly five times the original price, $2010 before shipping. Some further correspondence from Narishige involved thanks for our "introducing of the item [we] designed to other universities" and successive offers of possibly useful further modifications they could provide, ones not really needed and never incorporated (Addenda 5,6). Part of that correspondence was a very nice letter of thanks from the founder, Mr. Narishige, himself .

(In a neurobiology lab course taught for many years with another colleague recruited from Harvard, Doju Yoshikami, at least half a dozen Narishige MO-15 manipulators were deployed at student stations for electrophysiology experiments. Owing to budget constraints, only the original, unmodified MO-15 models could be afforded for student use, so the students had to learn the awkward, sawtooth X-Z stepwise approach to movements along the X-axis parallel to a culture dish.)

Mario's single-cell injections soon graduated to use of fancier, more expensive micromanipulators from the Leitz/Leica company, as illustrated in the talk he presented during the Nobel Prize ceremonies (Addendum 7). Even later, he used the electroporation technique for introducing DNA into cultured cells -- application of electric shocks temporarily rendering most cells in a culture dish permeable to large molecules, presumably by opening transient holes in their membranes. While that approach was much less efficient, on a per-cell basis, in introduction of the exogenous DNA into genomes of the recipient cells, it allowed easy application to very large numbers of cells at a single stroke. It thus greatly increased practical efficiency compared to painstaking manual injections into one cell at a time.

And, as a suitable end to this story, once Narishige had presumably become aware of Mario's changes of approach, the 'Okun-Capecchi Special MO-15' model disappeared from their catalog.

Larry Okun

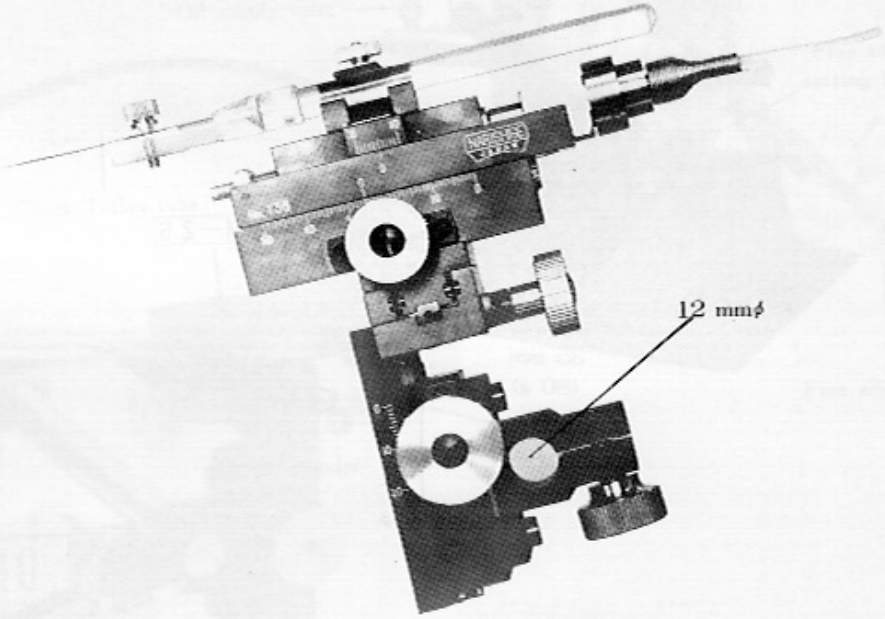
Addendum 1. Unmodified MO-15 micromanipulator.

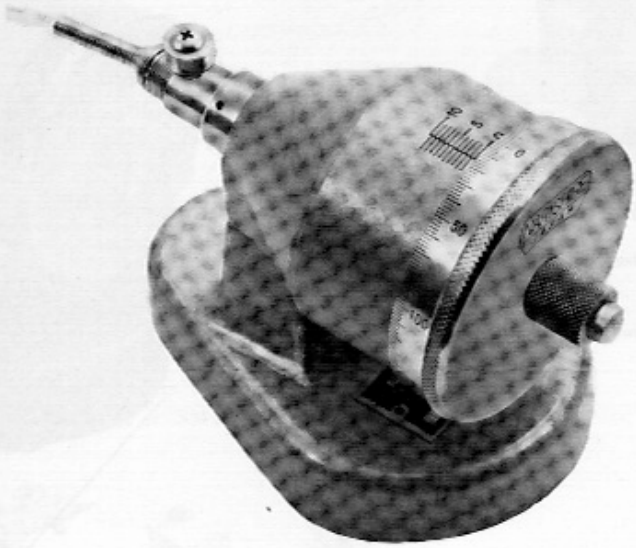
NARISHIGE
JAPAN

MO-15

Hydraulic Microdrive

This instrument, which has the hydraulic mechanism built into the X direction fine adjustment section of the MM-33 type micromanipulator, is a hydraulic microdrive that combines the smooth sliding mechanism of the MM-33 type micromanipulator's steel ball and stainless rail with the features of the hydraulic drive. This combination ensures accurate, vibration-free fine adjustment even under slight eccentricity and load.





X direction (fine adjustment) 10 mm
1 rotation 500 μm, minimum scale 2 μm
X direction 60 mm
Y direction 20 mm
Z direction 25 mm

Addendum 2. Original request to Narishige for modification of the MO-15, plus their reply (an invoice).

August 21, 1972

Narishige Scientific Instrument Laboratory
4-27-9 Minami-Karasuyama
Setagaya-Ku
Tokyo
JAPAN

Dear Sirs:

I would like to know if it would be possible to have a special model of the MO-15 made for me as shown in the attached diagram. This special model would be very useful for tissue-culture studies. The ball-joint would allow the axis of the hydraulic drive to be inclined downward toward a microscope stage at any angle, and the coarse bottom movements of the MM-33 would be arranged to allow manipulation of the inclined electrode in an X-Y plane without change of the electrode height.

The diagram shows a RIGHT-HAND special model. I would also want a second, LEFT-HAND version made with part (1) from a right-hand MM-33 and part (3) from a left-hand MO-15.

Your new catalogue lists the price of an ordinary MO-15 as U.S. $295. Please write to tell me the price of the special model I have described. Also, tell me if you have any questions about the design of the special model or if your engineers would recommend any changes of the design I have proposed.

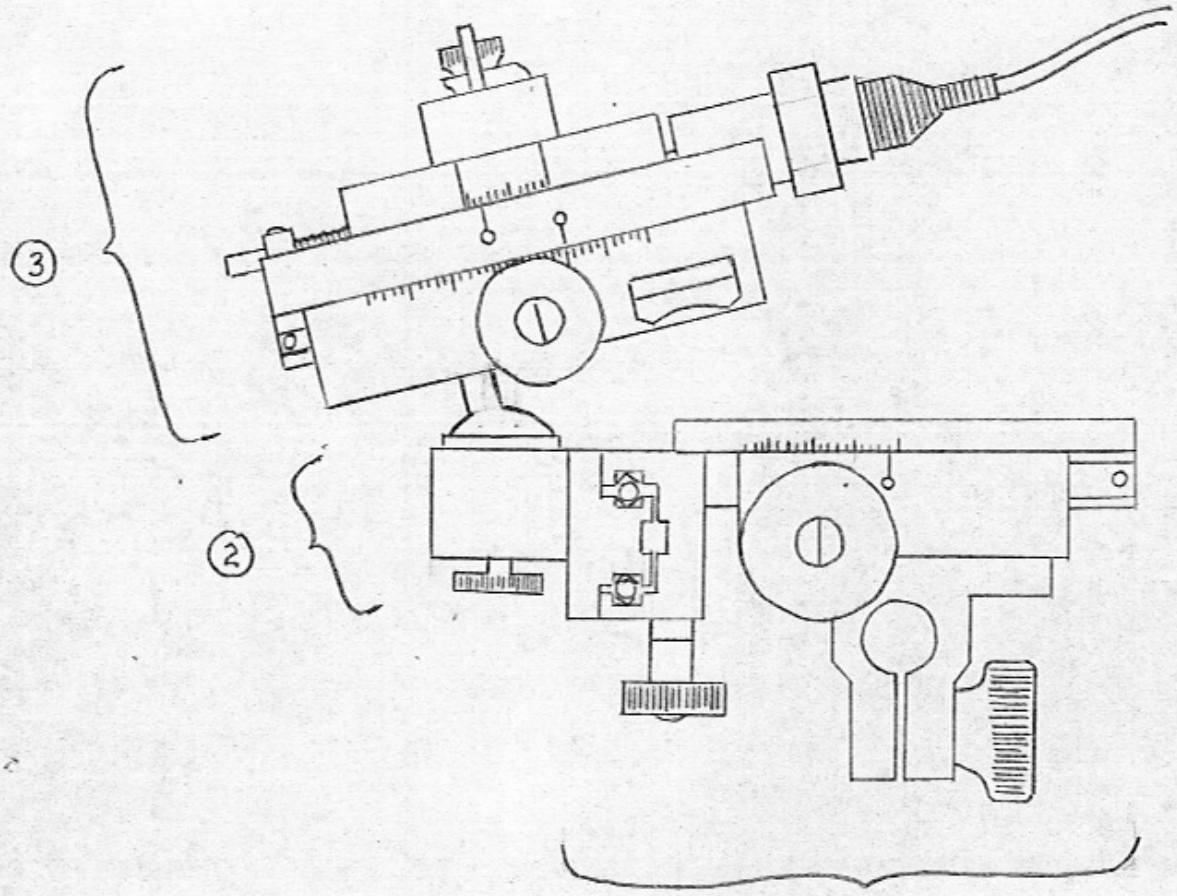
Sincerely yours,

Lawrence M. Okun
Assistant Professor

mak
Attachment

Proposed special model of MO-15 with ball-joint

Dr. L. M. Okun
Dept. of Biology
University of Utah
Salt Lake City Utah 84112
U.S.A.



For RIGHT-HAND special model:

① = Bottom ("Y" and "Z") movements of LEFT-HAND MM-33.
② = Ball-joint with screw-knob lock (as in BM-5).
③ = Top ("X") movements of RIGHT-HAND MM-33 with hydraulic micro-drive as in MO-15.

NARISHIGE SCIENTIFIC INSTRUMENT LABORATORY
1754-6, KARASUYAMA-CHO, SETAGAYA-KU
TOKYO, JAPAN

成茂科学器械研究所
本社 東京都世田谷区烏山町1754-6
27-9 4-CHOME MINAMIKARASUYAMA
SETAGAYAKU TOKYO JAPAN

INVOICE No. 72-149

TOKYO. Sept. 5, 1972

INVOICE of Micromanipulator shipped in good order and condition.

per Aircarft (parcel post) From Tokyo, Japan To Salt Lake city

By order of and for account and risk of The University of Utah Department of Biology

Under

Marks & Nos.	Quantity	Description	Price	Amount
A.D.D.	1 set	Micromanipulator type-MO-15 Special model	US$390.00	US$390.00
			Air parcel post charges	26.00
			C.&.F.	US$416.00

DELIVERY:Within 30 days after the your firm order.

NARISHIGE SCIENTIFIC INSTRUMENT LAB.
Eiichi Narishige
MANEGER E. NARISHIGE

Addendum 3. Mario's seminal microinjection paper, first page and page citing use of modified MO-15 micromanipulator

Cell, Vol. 22, 479–488, November 1980 (Part 2), Copyright © 1980 by MIT

High Efficiency Transformation by Direct Microinjection of DNA into Cultured Mammalian Cells

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Department of Biology
University of Utah
Salt Lake City, Utah 84112

Summary

Direct microinjection of DNA by glass micropipettes was used to introduce the Herpes simplex virus thymidine kinase gene into cultured mammalian cells. When DNA was delivered directly into the nuclei of LMTK$^-$, a mouse cell line deficient in thymidine kinase activity, 50–100% of the cells expressed TK enzymatic activity. In contrast, no TK activity could be detected when the DNA was injected into the cytoplasm. The number of injected LMTK$^-$ cells capable of indefinite growth in a TK$^+$ selective medium (that is, transformants) depended on the nature of the plasmid DNA into which the HSV-TK gene was inserted. One cell in 500–1000 cells which received nuclear injections with pBR322/TK DNA gave rise to a viable colony when grown in HAT medium (that is, a TK$^+$ selective medium). The transformation frequency increased to one in five injected cells when specific SV40 DNA sequences were also introduced into the HSV-TK plasmid. With the microinjection procedure transformation frequency was relatively insensitive to DNA concentration and did not depend on co-injecting with a carrier DNA. Most of the transformants were stable in *nonselective* medium as soon as they could be tested.

Introduction

Specific genes can be introduced into cultured mammalian cells by chromosome-mediated gene transfer (McBride and Ozer, 1973; Willecke and Ruddle, 1975) and by purified DNA-mediated gene transfer (Bacchetti and Graham, 1977; Maitland and McDougall, 1977; Wigler et al., 1977). The uptake and expression of both the metaphase chromosomes (Spandidos and Siminovitch, 1977; Miller and Ruddle, 1978) and purified DNA (Graham and van der Eb, 1973) is enhanced by the formation of a DNA-calcium phosphate precipitate. One in 10^5–10^7 treated cells becomes transformed by either chromosome- or DNA-mediated gene transfer. The rare transformant is isolated by biochemical selection.

In the initial transformation experiments using calcium phosphate precipitation to facilitate the uptake of purified DNA, the Herpes simplex viral thymidine kinase gene (HSV-TK) was transferred into LMTK$^-$, a mouse cell line deficient in thymidine kinase (Bacchetti and Graham, 1977; Maitland and McDougall, 1977; Wigler et al., 1977). This approach has been extended to the cellular genes for thymidine kinase (Wigler et al., 1978), adenine phosphoribosyl transferase (Wigler et al., 1979a) and hypoxanthine phosphoribosyl transferase (Graf, Urlaub and Chasin, 1979; Willecke et al., 1979).

For each of the above experiments a good selection procedure for isolating the transformants existed. It also became apparent that the transformation frequency was critically dependent on the particular cell line used as the recipient (Graf et al., 1979).

In an elegant set of experiments Wigler et al. (1979b) showed that nonselectable genes could be introduced into cultured mammalian cells by co-transformation with a unlinked but selectable gene. The nonselectable gene was mixed in a molar ratio of 1,000 to one with the selectable gene (HSV-TK) precipitated with calcium phosphate and layered onto LMTK$^-$ cells. More than 90% of the LTK$^+$ transformants contained multiple copies of the nonselectable gene.

In this study an alternative method of transferring purified genes into cultured mammalian cells is described. The DNA was directly injected into the nucleus using glass micropipettes (Diacumakos, 1973; Graessmann and Graessmann, 1976; Stacey and Allfrey, 1976). The transformation efficiency of the HSV-TK gene inserted into a number of different recombinant plasmids was compared. The transformants were characterized for the presence of HSV-TK enzymatic activity and for their stability in nonselective medium.

Results

Injection of pBR322/TK DNA

The microinjection experiments were initiated with two objectives in mind. The first was to determine the efficiency of DNA-mediated transformation obtained by microinjecting the DNA into cells and to compare this efficiency with that obtained with the more familiar calcium phosphate precipitation methods described by Bacchetti and Graham (1977), Maitland and McDougall (1977) and Wigler et al. (1977). The second objective was to attempt to discover the steps limiting the efficiency of the transformation process itself. DNA-mediated transformation of cultured mammalian cells can be divided into several steps including the DNA's entry into the cell, its transfer from cytoplasm to nucleus and its integration into the host genome. It seemed likely that microinjection of the DNA could be used to evaluate how much each step limits the frequency of transformation.

The experimental system chosen for these studies was the transfer of the Herpes simplex virus I thymidine kinase gene (HSV-TK) into a thymidine kinase deficient mouse fibroblast cell line, LMTK$^-$. The DNA injected was a purified preparation of E. coli plasmid pBR322 which carried the HSV-TK gene as an insert

cropipettes provides an alternative procedure for introducing genes into cultured mammalian cells. When appropriate recombinant plasmids are used, transformation frequencies approaching unity are obtained. With these high transformation frequencies the application of the technique to embryological problems becomes feasible.

Experimental Procedures

The methods used for culturing the cells, preparing cell extract and autoradiography have been described in detail elsewhere (Sharp, Capecchi and Capecchi, 1973; Wahl, Hughes and Capecchi, 1975; Capecchi et al., 1977).

Microinjection

The recipient cells were grown on small glass slides (10 × 10 mm). The solution of macromolecules was injected into the cells via glass micropipettes having tip diameters ranging from 0.1 to 0.5 μ. The pipettes were prepared from glass capillaries (Omega Dot Tubing, 1.2 mm OD, W. P. Instruments) on a Model P77 Brown-Flaming micropipette puller (Sutter Instruments). Injections were carried out under direct visual control on a fixed stage of an inverted phase contrast microscope (Leitz Diavert, 400X). Movement of the micropipettes was controlled with micromanipulators (Narishige MO-15, modified) which have a hydrolic microdrive along the pipette axis. The fluid containing the macromolecules is forced into the cells under constant pressure supplied by a Hamilton threaded plunger syringe (Model 87000). The amount of fluid injected into each cell was controlled with moderate precision (within a factor of two) by visually monitoring changes in the cellular refractive index as the fluid enters the cell and regulating the time that the micropipette remained in the cell. The average volume (10–20 femtoliters) injected into each cell was determined by injecting ^{3}H-dTTP (10 μc/μl) into five thousand cells, washing the cells with PBS and then measuring the radioactive content by liquid scintillation counting. For L cells this volume corresponds to 1–2% of the cell volume. With practice one can attempt injections into 500–1000 cells per hour with a successful transfer of material being assured in 50–100% of them. Similar procedures for injecting macromolecules into cultured mammalian cells have been described by Graessmann and Graessmann (1976), by Diacumakos (1973) and by Stacey and Allfrey (1976).

Preparation of Recombinant Plasmids

Restriction enzymes and T4 DNA ligase were obtained from BRL and New England Biolabs and used under conditions recommended by the vendors. Plasmid DNAs were isolated from cultures of E. coli HB101, grown to saturation in NZYPD medium (per liter: 5 g NaCl, 2 g $MgCl_2$, 10 g NZ amine A, 1 g caseamino acids and 5 g yeast extract). The cells were lysed with 0.03 M NaOH, 0.003 M EDTA and 0.5% SDS. The chromosomal DNA was precipitated with 1 M NaCl and 3% PEG. Following the addition of NaCl and PEG the extracts were incubated for 2 hr at 4°C and centrifuged at 20,000 × g for 30 min. The supernatants containing the plasmid DNA were extracted twice with chloroform-phenol. The RNA was removed by gel filtration on a Biorad A50 column.

SV40 DNA was isolated from lytically infected TC7 cells and purified by the procedure of Trilling and Axelrod (1970). The HSV-1 Bam HI fragment which contains the HSV-TK gene was purified from pBR322/TK DNA by digestion to completion with Bam HI and fractionation on a 0.9% agarose gel.

SV40 was inserted into pBR322 by digesting both to completion with Eco RI. The pBR322 DNA was then treated with alkaline phosphatase [(pH 9) 68°C for 30 min] to prevent self ligation. After ligation of pBR322 and SV40 DNA with T4 ligase it was used to transform E. coli strain HB101. The resulting ampicillin resistant colonies were screened for recombinant plasmids on agarose gels. pBR322/SV-O+T/TK was prepared as described above by inserting the purified HSV-Bam HI fragment into pBR322/SV40. Following ligation the DNA was used to transform E. coli HB101. 90% of the ampicillin-resistant colonies contained the desired recombinant plasmid. pBR322/SV-O/TK was prepared from pBR322/SV-O+T/TK DNA by partial hydrolysis with Hind III followed by ligation with T4 ligase.

Acknowledgments

I would like to thank L. Fraser and R. Myers for expert technical assistance and E. Sparks and J. Harshman for help in the initial phases of these experiments. I also wish to express my gratitude to L. Okun for many thoughtful and encouraging discussions concerning the setting up of the microinjection apparatus. Technical advice concerning cloning procedures from P. Luciw is gratefully acknowledged. The DNA-mediated transformation studies using the calcium phosphate precipitation method were carried out by P. Barry.

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Received August 11, 1980; revised September 5, 1980

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Addendum 4. Narishige catalog page with special MO-15-S, modified as in the original request.

NARISHIGE
JAPAN

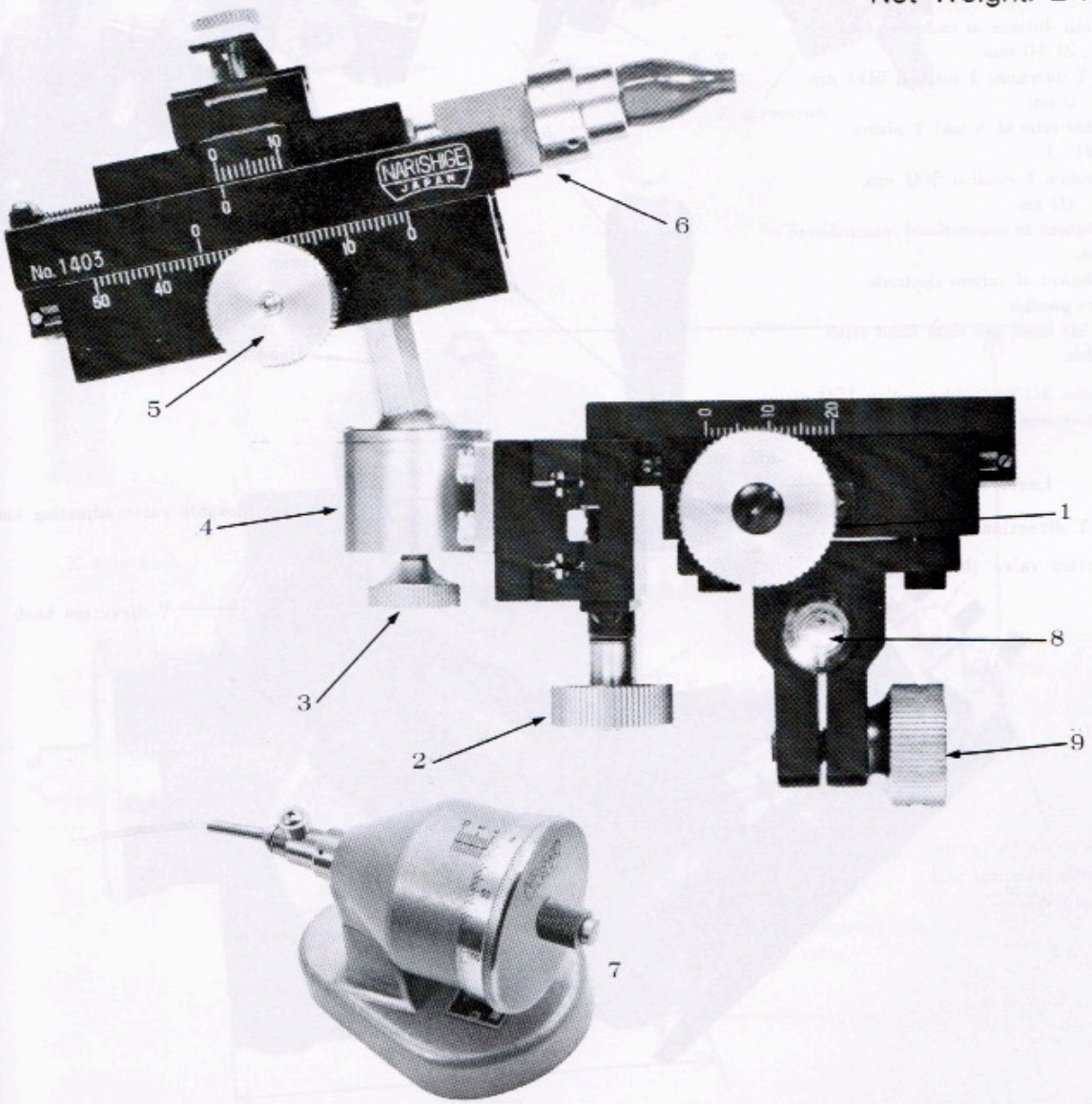
MO-15-S

This instrument is a special model of the MO-15 with a ball joint. The redesign is by Dr. L. M. Okun and Dr. Mario R. Capecchi (Dept. of Biology, Univ. of Utah) with following special functions:

1. Range is 25 mm in X direction (left and right).
2. Range is 20 mm in Y direction (front and back).
3. Holding screw for the ball joint.
4. Ball joint.
5. Range is 50 mm in D direction (depending on orientation).
6. Range is 10 mm in D direction (depending on orientation).
7. Fine adjustment of hydraulic movement.
8. Clamp hole 12 mm or 1/2 inch.
9. Clamp screw.

This instrument has been used to inject chemicals directly into blood vessels, cultured cells, etc.

Net Weight: 2 kg



Price list Dec '83

PRODUCT NO.	PRODUCT DESCRIPTION	PRICE $ USA	PAGE NO.
MO-8	Hydraulic microdrive	1284.00	21
MO-9-A	Hydraulic microdrive	2055.00	74
MO-9-B	Hydraulic microdrive	2184.00	74
MO-10	Hydraulic microdrive	963.00	21
MO-11	Single dimensional hydraulic microdrive	1092.00	22
MO-15	Hydraulic microdrive built on MM-3 micromanipulator	1413.00	23
MO-15-S	Same as above but with ball joint movement	2010.00	24
MO-81	Stepping hydraulic microdrive, electronic digital counter	3854.00	77
MO-90	Hydraulic microdrive	2569.00	74
MO-95-A	Skull mounting type hydraulic microdrive 40mmZ	2119.00	75
MO-95-B	Skull mounting type hydraulic microdrive 50mmZ	2248.00	75
MO-99	Skull mounting type hydraulic microdrive	1605.00	76
MO-102-R	Hydraulic micromanipulator with Joy stick, Right hand use	3220.00	25
MO-102-L	Same as above but left hand use	3220.00	25
MO-103-R	3 Dimensional hydraulic microdrive, Right hand use ...	2575.00	26
MO-103-L	Same as above but left hand use	2575.00	26
MO-104-R	Same as MO-102 but with four degrees of freedom.....	4305.00	27
MO-104-L	Same as above	4305.00	27
MO-951	Skull mounting type hydraulic microdrive	4753.00	72
MP-1	Micromanipulator Peterfi-type on tilting base, complete set of Right and Left hand use with steel base (530 x 380 x 28mm)	2955.00	1
MP-1-R	Micromanipulator Peterfi-type Right hand use only	1397.00	1
MP-1-L	Same as above but left hand use only	1397.00	1
MP-1-S	Micromanipulator Peterfi-type without steel base plate	2794.00	1
MP-2	Same as MP-1 but with a M-1 substage	3533.00	2
MP-2-R	Peterfi-type micromanipulator with M-1 substage (Right hand use only)	1686.00	2
MP-2-L	Same as above but left hand use only	1686.00	2
MP-2-S	Same as MP-1-S but with M-1 substage	3372.00	2
MP-6	Micromanipulator - pencil type	578.00	
MP-6-S	Same as above but mounted on Arm	578.00	
MQ-1	Accessories for inverted microscope		
	A sets	514.00	28
	B sets	578.00	28
	C sets	642.00	28
MT-5	Minature micromanipulator	770.00	9
MZ-9	High resolution micromanipulator	578.00	15
MZ-10	High resolution micromanipulator	2569.00	16
PA-81	Glass micro-electrode puller	2569.00	36
PD-5	Horizontal type double barrelled glass micro-electrode puller	1927.00	34
PE-2-M	Vertical type glass micro-electrode puller adjustable	2569.00	32
PG-1	Glass micro-electrode puller manual operation type heating coil	1477.00	31
PN-3	Horizontal type micro-electrode puller	1480.00	33

Addendum 5. Narishige letter with thanks and offer of added vertical movement, plus my reply.

NARISHIGE SCIENTIFIC INSTRUMENT LABORATORY LTD.

27-9, MINAMI-KARASUYAMA 4-CHOME, SETAGAYA-KU, TOKYO, JAPAN.

TEL. (03) 308-6233 - CABLE: NARISHIGE.

Nov. 13th, 1981.

Dr. L. M. Okun
Dept. of Biology
University of Utah
Salt Lake City, Utah 84112
U.S.A.

Sear Sir.

First of all we would like to say thank you very much for the introducing of the item MO-15 you designed to other universities, but we think that this item has some difficulty to operate because as you know this instrument has no vertical movement factor and as the result of this it might be difficult to get the focus.

It would be our pleasure, if we will be able to get your opinion for this matter, because we would like to introduce it to other doctors too for their convenience.

Thank you very much in advance.

We remain.

With Best Regards.

Sincerely Yours.

Export Manager.

Mr. Narishige.

THE
UNIVERSITY
OF UTAH

DEPARTMENT OF BIOLOGY
201 BIOLOGY BUILDING
SALT LAKE CITY, UTAH 84112
801-581-6517

January 12, 1982

Mr. Narishige, Export Manager
Narishige Scientific Instr. Lab
27-9 Minami-Karasuyama
4 Chome
Setagaya-Ku
Tokyo, JAPAN

Dear Mr. Narishige:

I am writing to answer your questions about the special version of the MO-15 micromanipulator (the "L.M. Okun version") that I designed in 1972. (A copy of the original design drawing is enclosed.) This design has been very useful in my own electrophysiological studies (intracellular recording from neurons in cell cultures), and it has also been used very successfully by a colleague to inject DNA into the nuclei of cultured cells (Capecchi, 1980 Cell 22:479).

The special advantages of the design are as follows:

1. Straight (unbent) micropipettes can be advanced and withdrawn in the direction of their tips smoothly and by remote control without communicating vibration to the preparation on the microscope stage. This is extremely useful for penetrating cells in cultures, especially for penetrating many cells in succession.

2. The axis of the pipette (along which it is advanced and withdrawn) can be inclined (tilted) at a convenient angle with respect to the microscope stage by use of the ball-joint. It would be even more useful in some applications if the ball-joint permitted a steeper maximum angle of inclination than it now does, as it could, for example, if there were a notch (cut-out) that allowed the ball-post to tilt further forward.

3. The tilted pipette can be moved easily in the x-y plane without changing the distance of the tip from the microscope stage. This is not possible with the standard MO-15 since when that manipulator is tilted, the "z" and "x" controls must be used together in an awkward way to move the tip along an x axis parallel to the stage.

The absence of vertical control in the design is not important for most applications. Initial adjustment of the distance of the tip from the microscope stage (focus) is easily achieved by use of the coarse and fine (hydraulic) drives along the pipette axis and the easy control of the tip's position in the x-y plane. A vertical control might be useful in some cases but the extra expense of adding it is not likely to be worthwhile for most users.

(continued)

One original advantage of the manipulator was relatively low cost. This is no longer the case since the price I recently paid for a new one was three times the price I paid in 1972. If you could reduce the price without changing the design or quality, I am sure the item would be even more popular.

I hope these comments are helpful to you.

Sincerely yours,

Lawrence M. Okun
Associate Professor of Biology

LMO:tk

Addendum 6. Narishige offer of added angled block, plus my reply.

NARISHIGE SCIENTIFIC INSTRUMENT LABORATORY LTD.

27-9, MINAMI-KARASUYAMA 4-CHOME, SETAGAYA-KU, TOKYO, JAPAN.

TEL. (03) 308-8233 · CABLE: NARISHIGELABO

Jan. 22th, 1982

Dr. Lawrence M. Okun
Associate Professor of Biology
Department of Biology
University of Utah
Salt Lake City, Utah 84112
U.S.A.

Dear Sir.

Thank you very much for your letter dated Jan.12, 1982.
Your advice is very useful for us to improve the Model MO-15-S along your idea.
We are tring to improve the part of MO-15-S you have indicated as the picture shows.
Please find it and if this improvement is not suitable to your idea, please inform us.
As for this matter, we will change your instrument as you like in free of charge, according to your experiments.
The reason why the price of this instrument is high is we have to use two MM-33 (left-hand, right hand) and adding to this the amount of producing in this model is not so many at present.
In the future there is a possibility to reduce the cost according to the amount.
It would be our pleasure to accept your further advice for this matter.
We remian.

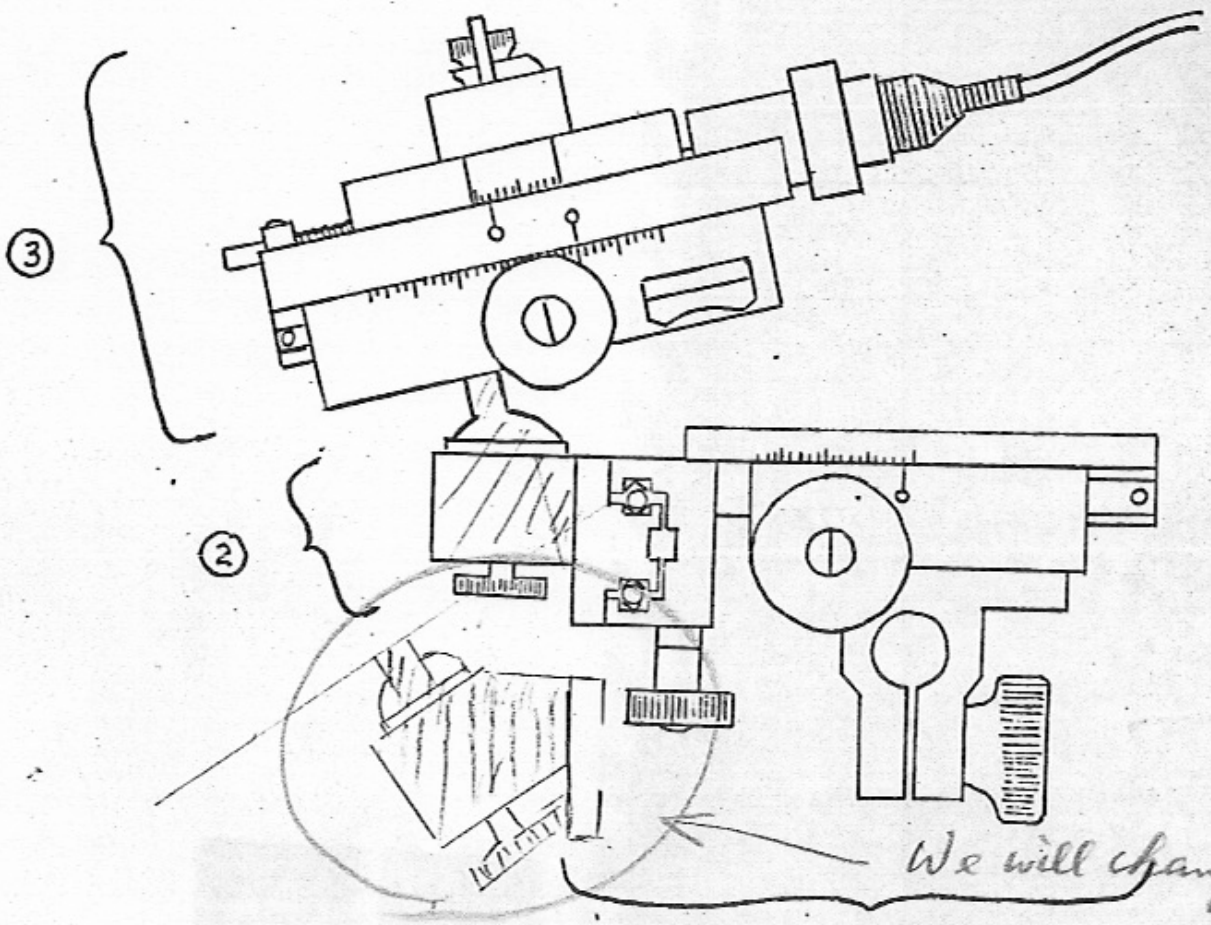
With Best Regards.

Yours Sincerely.

Mr. Narishige
President

posed special model of MO-15 with ball-joint

Dr. L. M. Okun
Dept. of Biology
University of Utah
Salt Lake City Utah 84112
U.S.A.



For RIGHT-HAND special model:

① = Bottom ("Y" and "Z") movements of LEFT-HAND MM-33.
② = Ball-joint with screw-knob lock (as in BM-5).
③ = Top ("X") movements of RIGHT-HAND MM-33 with hydraulic micro-drive as in MO-15.

March 29, 1982

Mr. Narashige, President
Narishige Scientific Instr. Lab
27-9 Minami-Karasuyama
4 Chome
Setagaya - Ku
Tokyo, Japan

Dear Mr. Narashige:

I am sorry it has taken me so long to answer your last letter.

Your design of an angled ball-joint block for the special MO-15 model seems very good. In fact, we had designed something similar to be used with the manipulators we have (see drawing). It is an intermediate block to be used between the ball-joint cup that we have and the manipulator itself. The holes drilled in it allow mounting of the ball-joint cup as shown without need for any new holes in the manipulator. The angle we have chosen allows the manipulator to reach horizontal position at its extreme "backward" tilt.

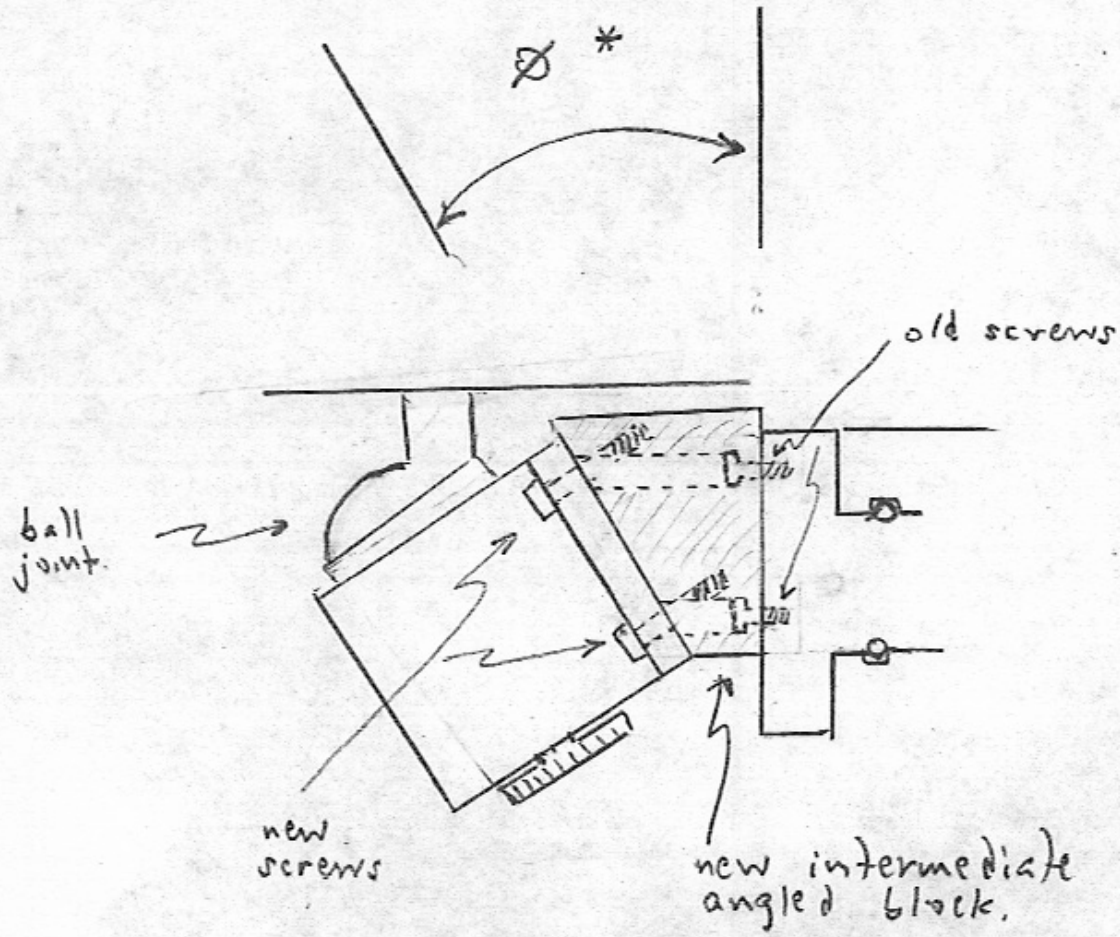
I hope this is helpful to you.

Sincerely yours,

Lawrence M. Okun
Assoc. Professor of Biology

LMO:mv
Enclosure

Angled intermediate block for Special MD-15 model:



angle Ø to allow horizontal position at extreme "backward" tilt (or shown)

Dr. L.M. Okun
Dept. of Biology
University of Utah
Salt Lake City, Utah 84112
USA.

Addendum 7. Microinjection apparatus as shown in Mario's Nobel Lecture.

from 'Gene Targeting 1977-Present'
Nobel Lecture, December 7, 2007
Mario R. Capecchi