膜片钳技术基本知识与常见问题

膜片钳设备组成及要求

膜片钳放大器

EPC系列（Heka)

Axon系列





HEKA EPC-10 . 8 . 7系列以及软件包

HEKA EPC-10 single/double/triple.EPC-10系列

方便快捷的快慢电容自动补偿
所有仪器操作只需要鼠标点击
软件构成的膜电容检测放大功能
灵活的刺激信号编辑功能
兼容的钙离子荧光检测功能
单探头，双探头，三探头多种选择

AXON，NPI公司膜片钳放大器，接口以及软件



Axopatch 200B



Multiclamp700A

Axopatch 200B,Multiclamp700A放大器用于多种细胞电流钳和电压钳纪录



Axopatch 2B

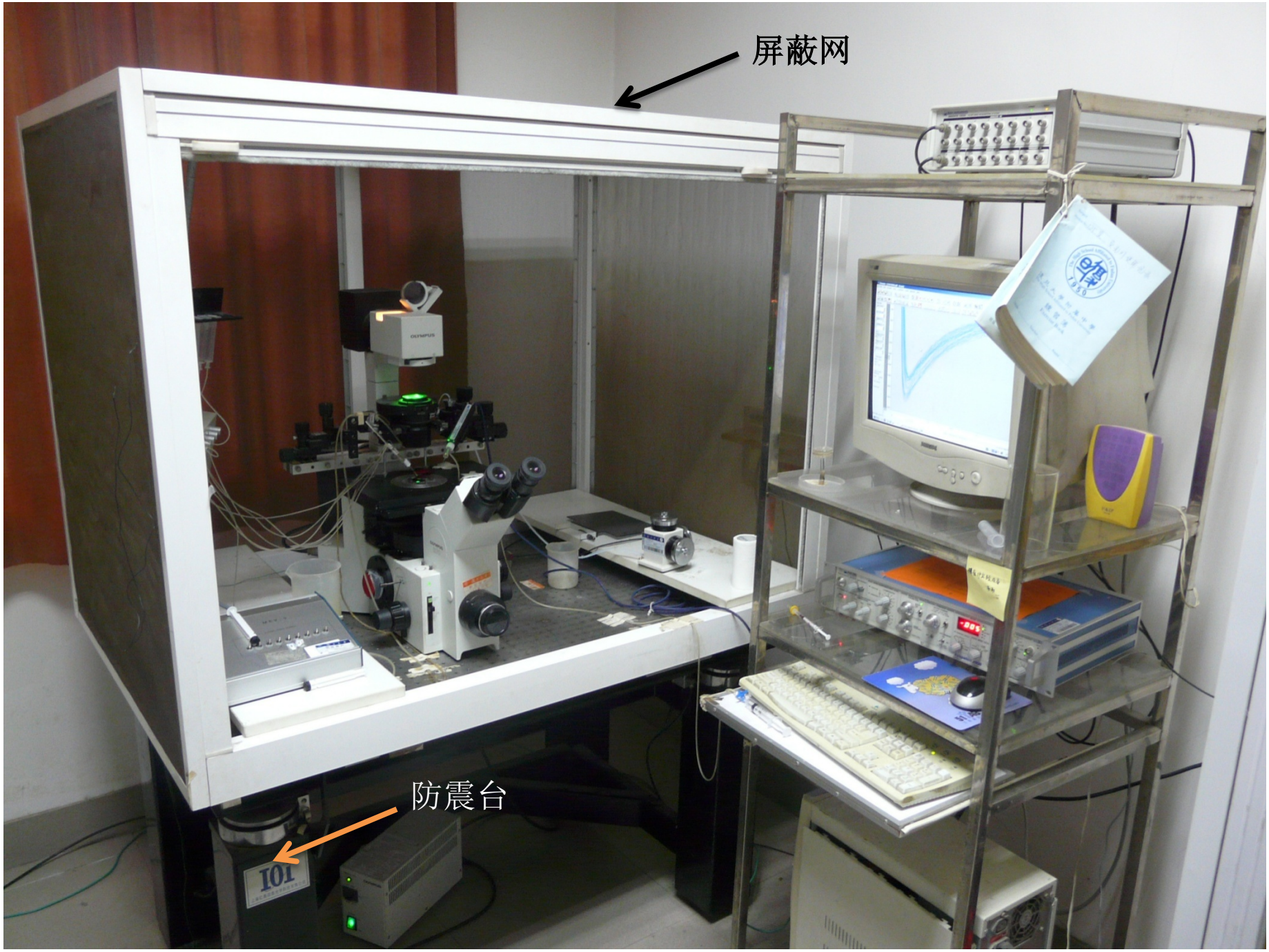


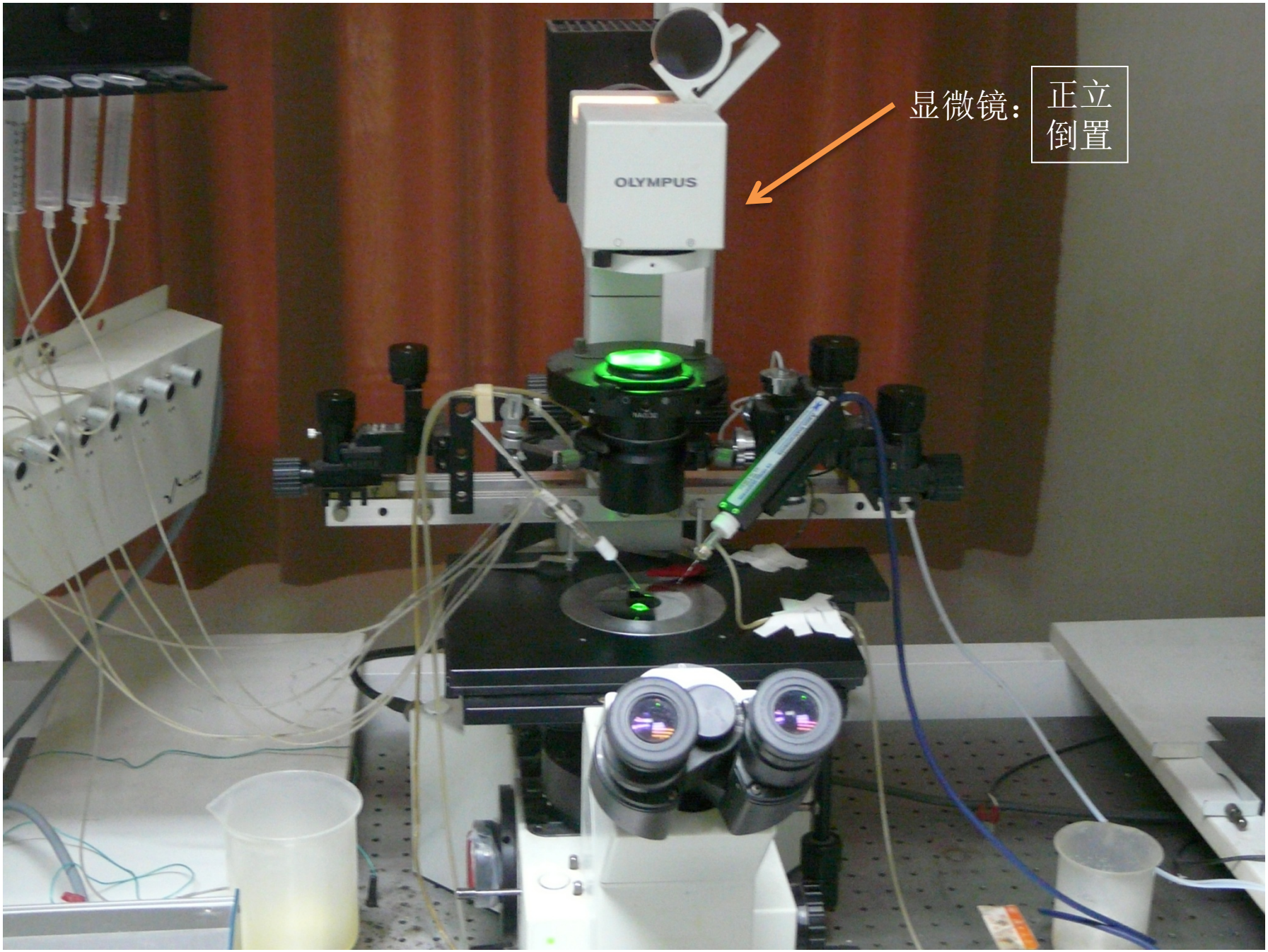
SEC Series



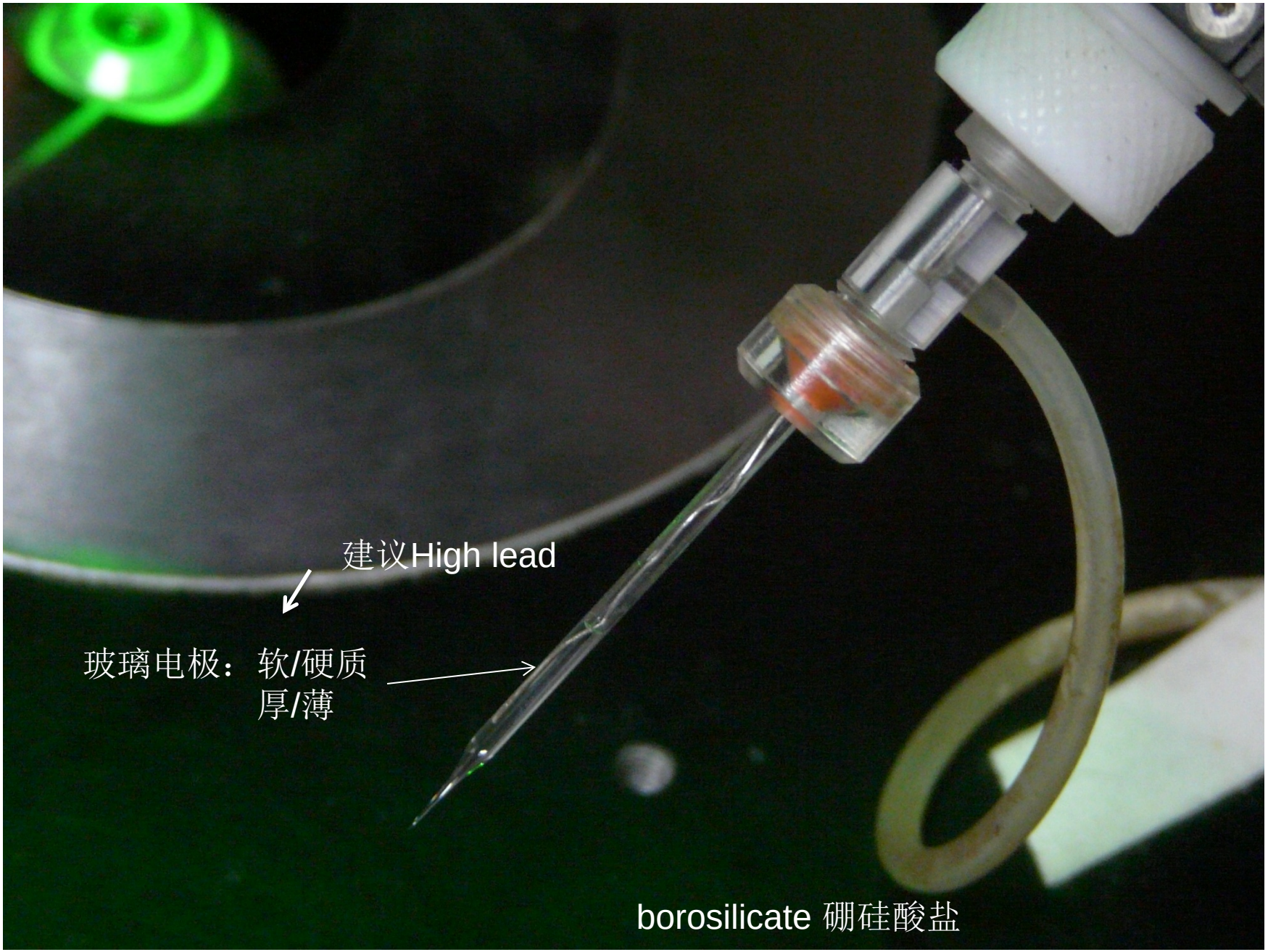
Geneclamp500B

Axopatch 2B放大器用于细胞内纪录，电压钳，电流钳
Geneclamp500B用于大细胞的胞内纪录，电压钳，电流钳
SEC型放大器用于多种细胞电流钳和电压钳纪录


屏蔽网
防震台
I01


显微镜：
正立
倒置
OLYMPUS


1
2
3
4
5
6
SHUTTER


建议High lead
玻璃电极：软/硬质
厚/薄
borosilicate 硼硅酸盐

Borosilicate Standard Wall with Filament



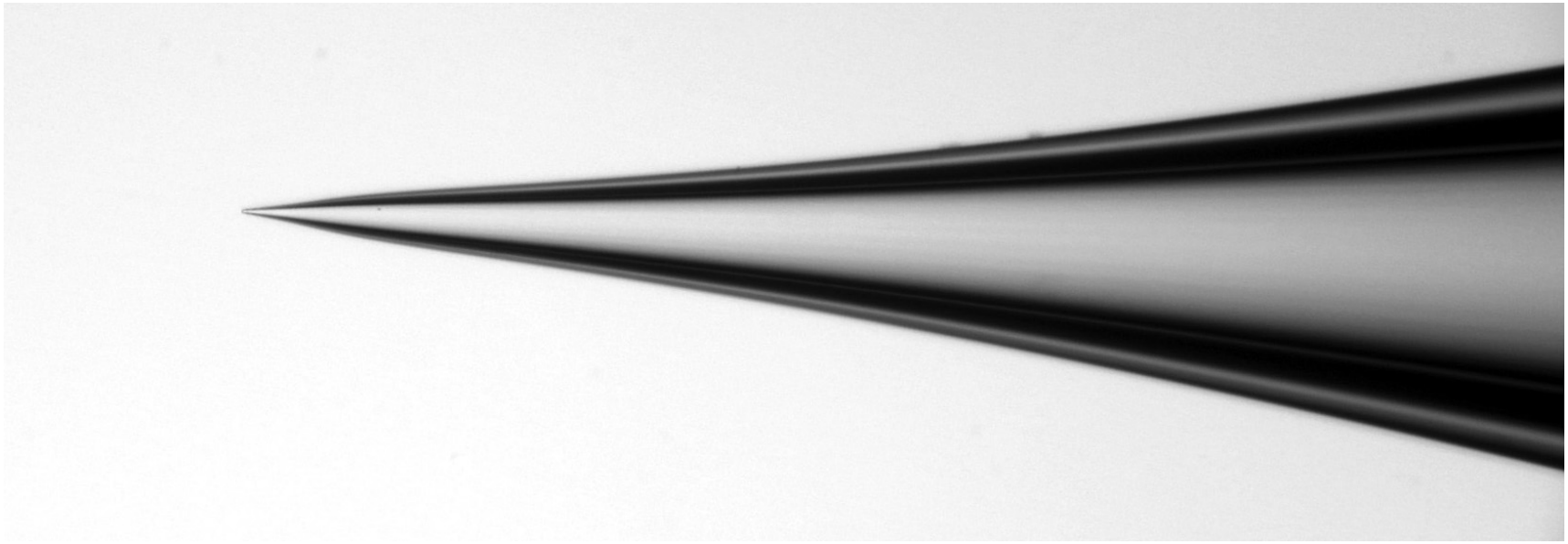


P-97 Pipette Puller

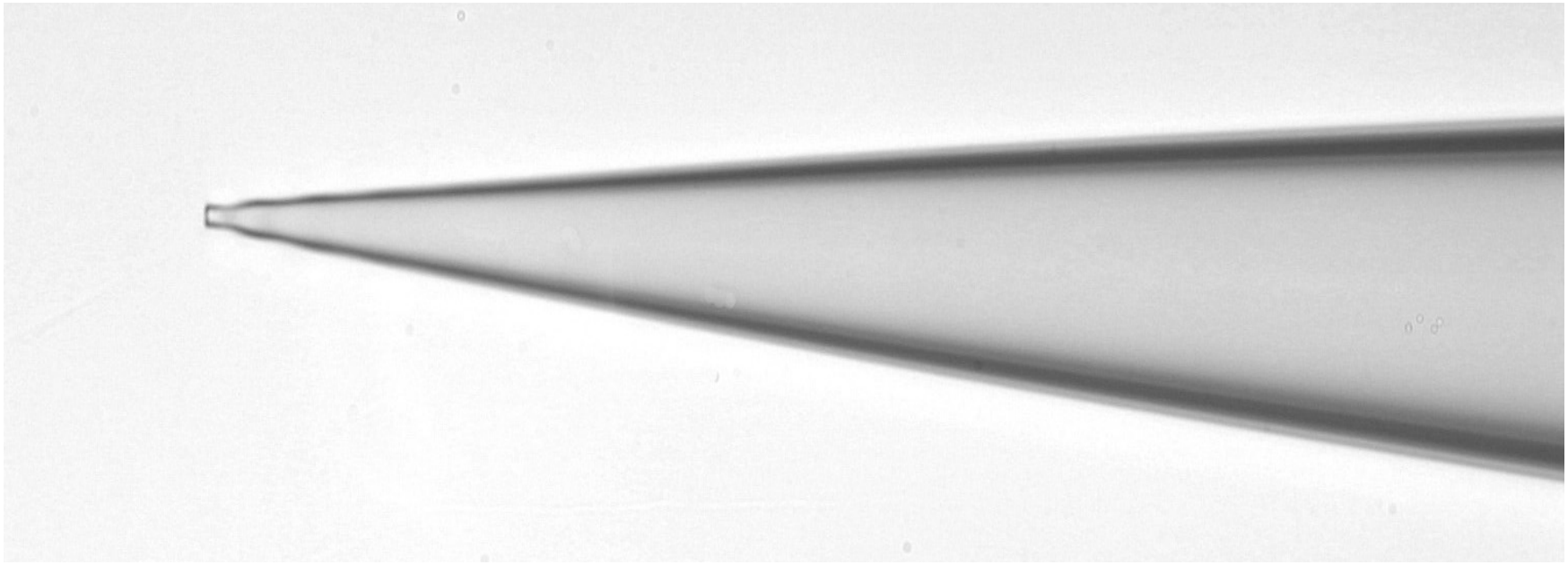


**New* P-1000 Pipette Puller

100X

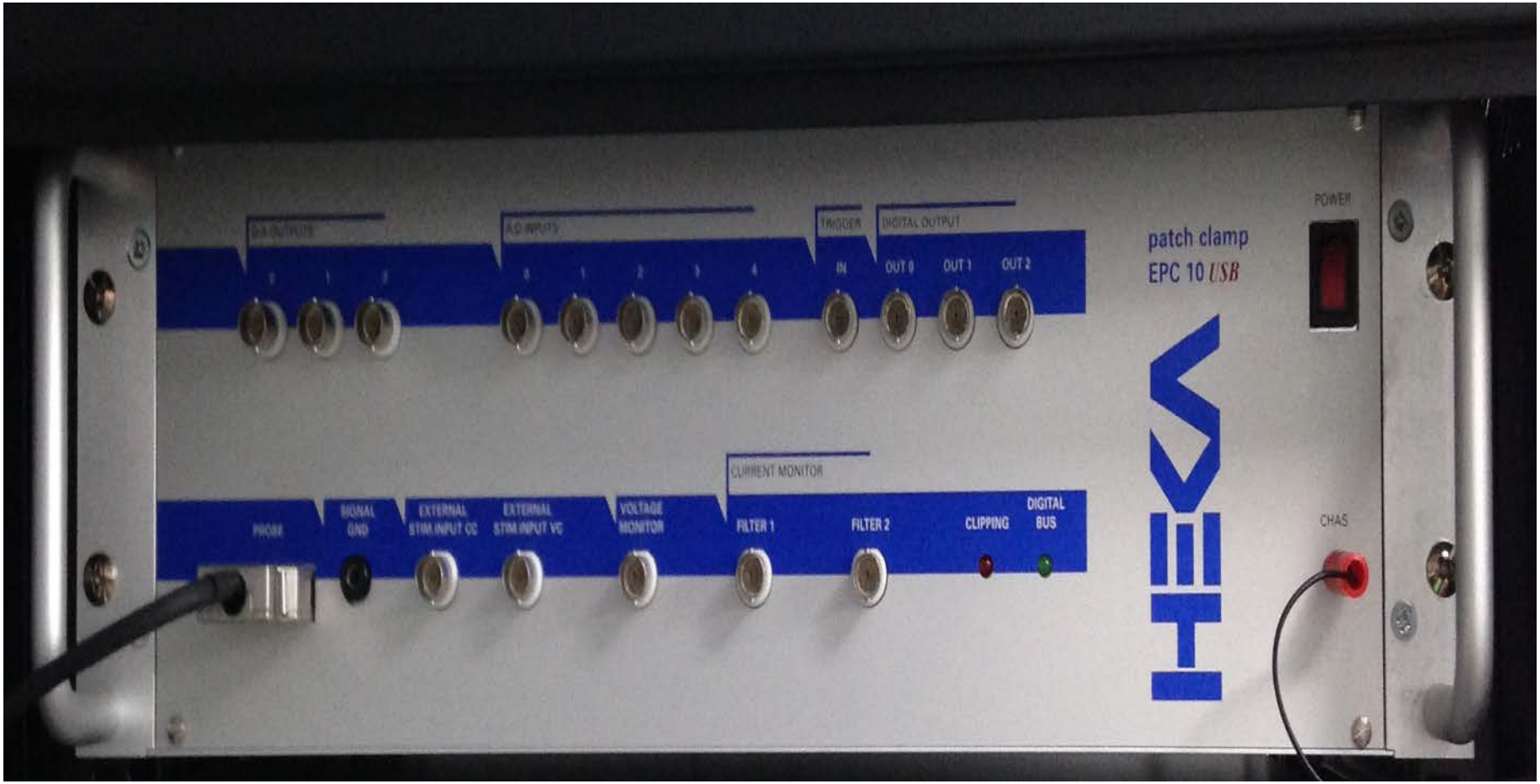


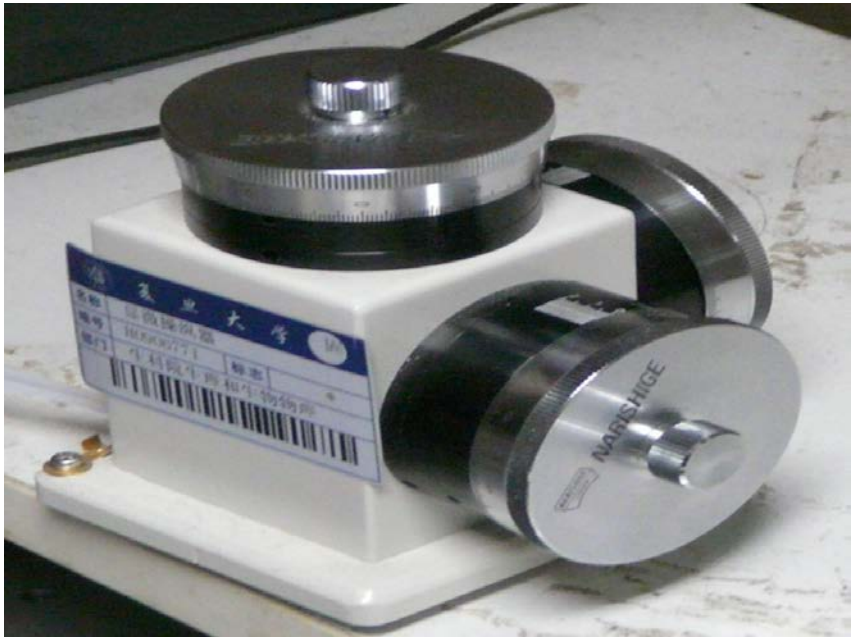
400X




放大器
Axon CNS
SERIES RESISTANCE COMP
WHOLE-CELL PARAMETERS
COMMANDS
METER
CONFIG
SCALED OUTPUT
ZAP
POWER
PIPETTE CAPACITANCE COMPENSATION
MODE
Axopatch 200B


Axon Instruments
DIGIDATA 1322A
16-BIT DATA ACQUISITION SYSTEM
POWER
READY
ANALOG IN
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
ANALOG OUT
0
1
DIGITAL OUT
0
1
2
3
TRIGGER IN
TAG
START


D-A OUTPUTS
A-D INPUTS
TRIGGER
DIGITAL OUTPUT
IN
OUT 0
OUT 1
OUT 2
patch clamp
EPC 10 USB
POWER
PHOTO
SIGNAL GND
EXTERNAL STIM.INPUT CC
EXTERNAL STIM.INPUT VC
VOLTAGE MONITOR
CURRENT MONITOR
FILTER 1
FILTER 2
CLIPPING
DIGITAL BUS
CHAS
HEKA


NARISHIGE


MP-225

基本知识

细胞的离子组成

Table 2.1 Intracellular and extracellular distribution of the main ions found in animal fluids

Ion	Intracellular range (mM)	Extracellular range (mM)
Na^+	5–20	130–160
K^+	130–160	4–8
Ca^{2+}	50–1000 nM[a]	1.2–4
Mg^{2+}	10–20	1–5
Cl^-	1–60	100–140
HCO_3^-	1–3	20–30

[a] Given as nanomolar rather than millimolar.

细胞内外液：配方以及渗透压（K通道）

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[a] Given as nanomolar rather than millimolar.

Bath solution containing (in mm): NaCl 125, KCl 2.5, HEPES10, MgCl 1, tetrodotoxin 0.001, TEA Cl20, glucose 10 (pH adjusted to 7.4 using NaOH)

internal solution containing (in mm): **K gluconate 135**, KCl 10, HEPES 10, CaCl2 1, MgCl21, **EGTA 10** (pH adjusted to 7.3 using **KOH**)

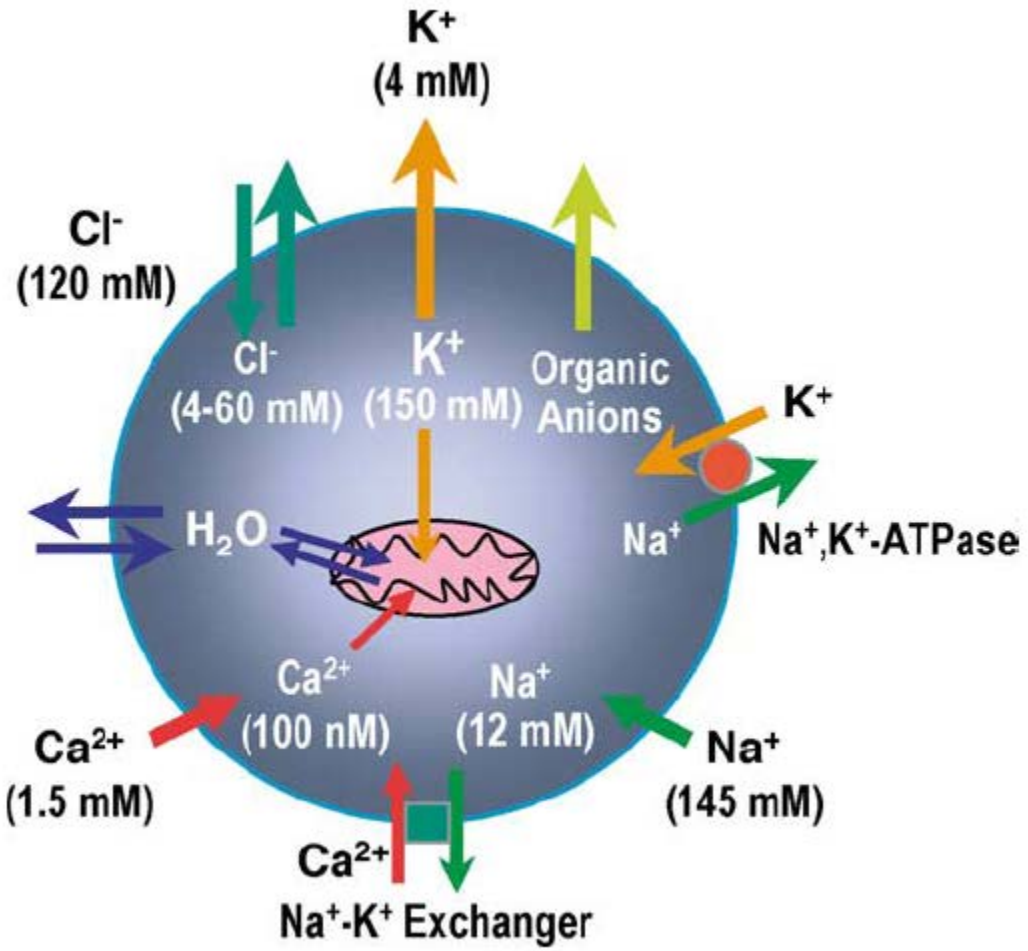
渗透压：外液通常大于内液20左右
如果内外液渗透压严重失衡可用蔗糖平衡

钙通道（举例）

External solution (inmM): 5 CaCl2, 156 **tetraethylammonium (TEA) chloride**, and 10 HEPES, glucose 10， pH adjusted to 7.4 with **TEA-OH**.

The internal pipette solution contained the following (inmM): **125 CsCl**, 10 EGTA, 2 CaCl2, 1 MgCl2, **4 Mg-ATP, 0.3 Na3GTP**, and 10 HEPES, pH adjusted to 7.2 with **CsOH**

细胞的极性



-30~-90mV

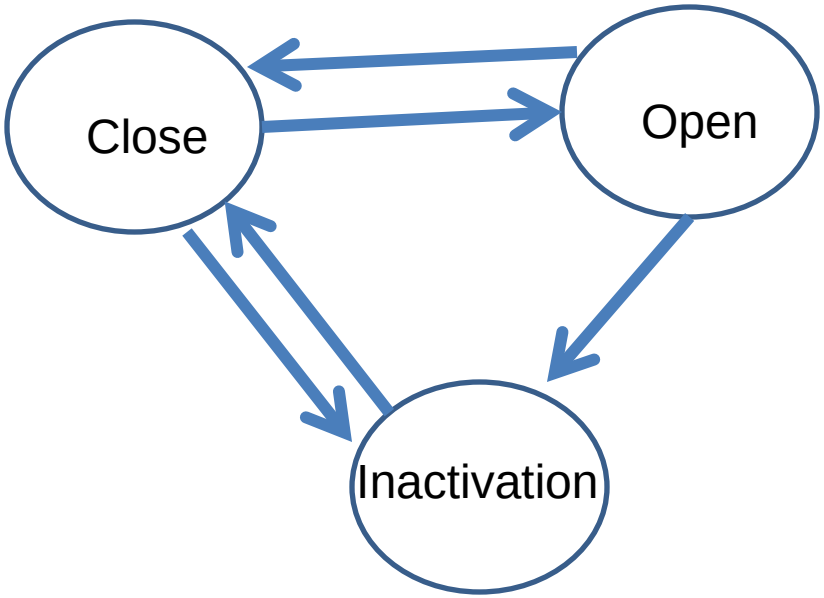
电压钳（voltage clamp）

控制电位测量电流变化

电流钳（current clamp）

控制电流测量电压变化

电压门控离子通道的特征



Activation depolarization -80------20

Inactivation

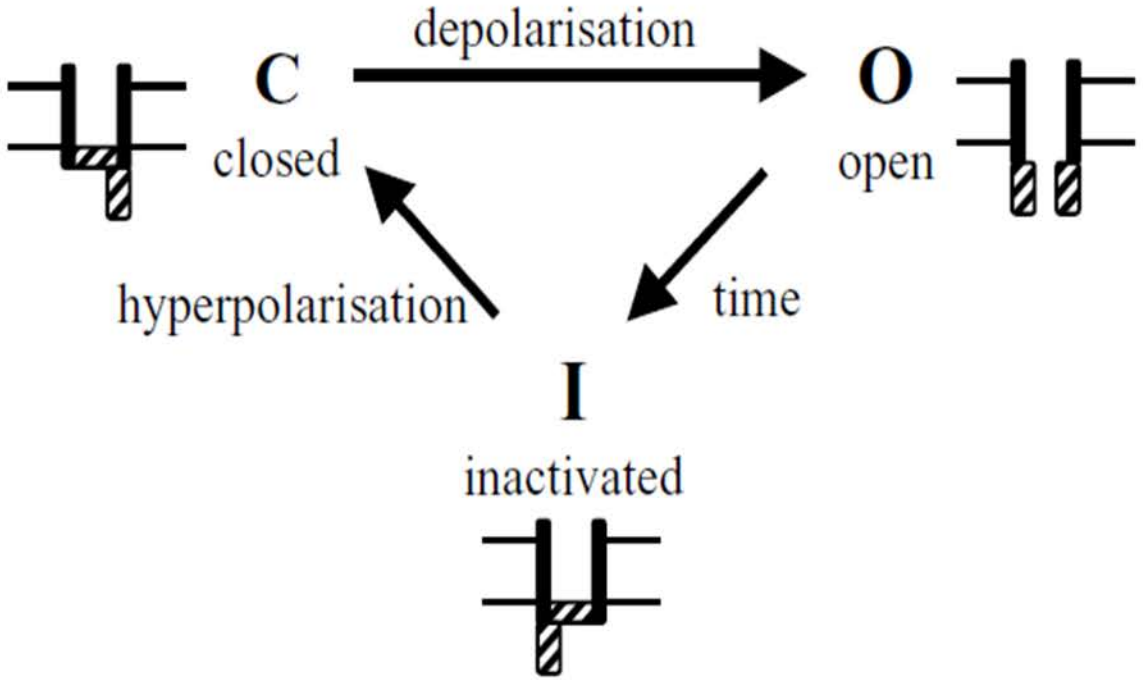
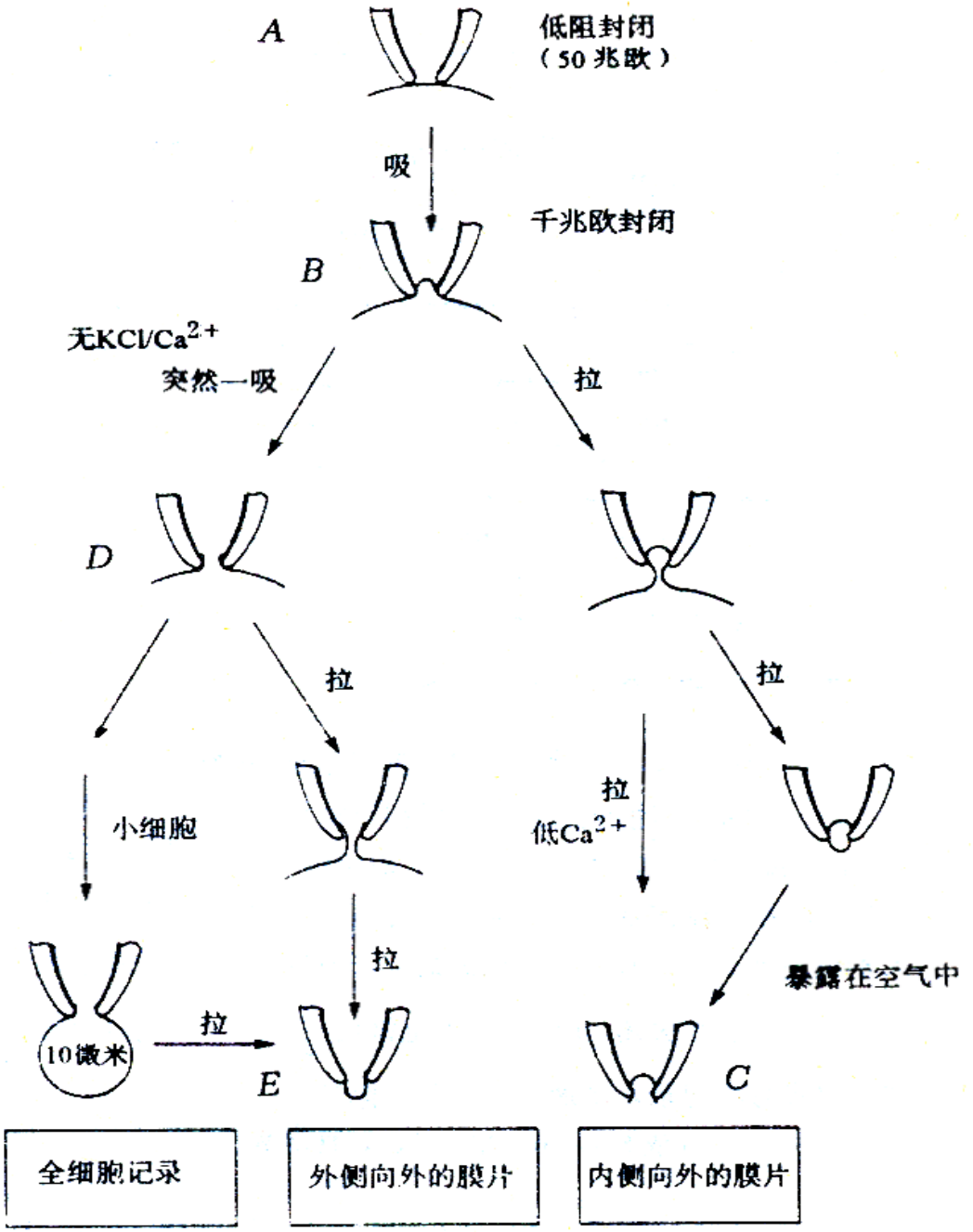


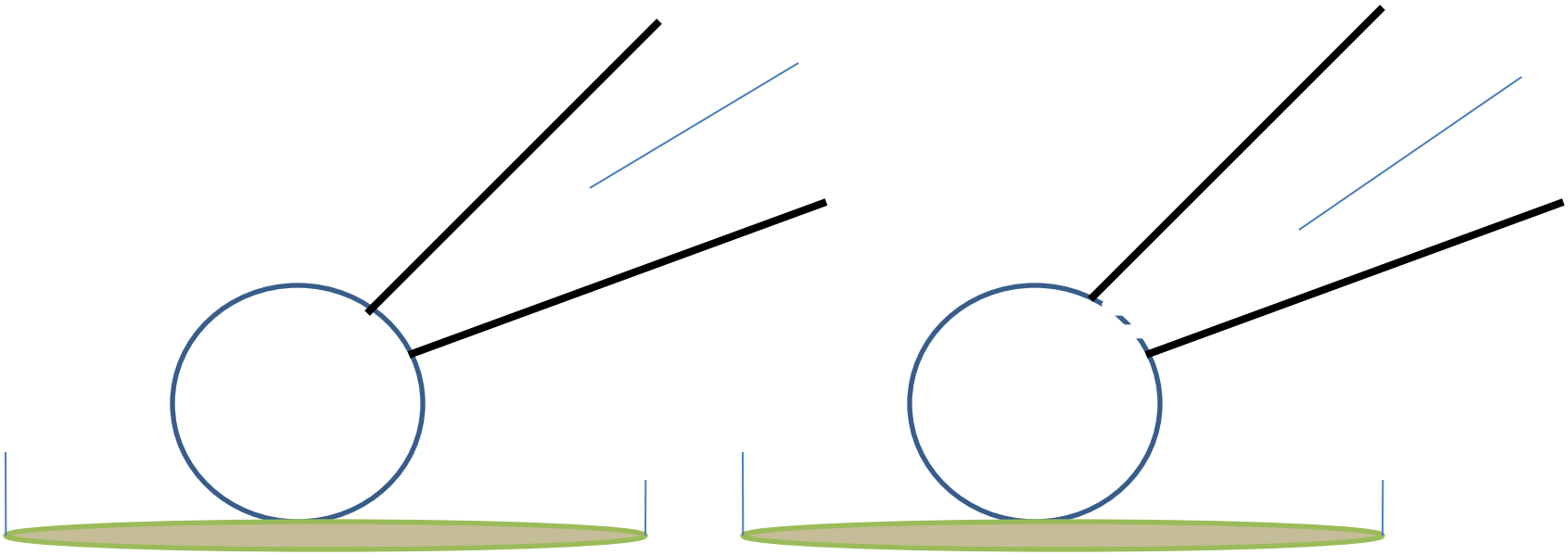
Figure 5.18 A sodium channel has separate activation and inactivation gates operated by membrane voltage and time

膜片钳记录的主要模式

- 细胞吸附模式 **(Cell-attach)**
- 全细胞记录模式 **(whole-cell recording)**
- 外侧向外的膜片 **(outside －out mode)**
- 内侧向外的膜片 **(inside-out mode)**



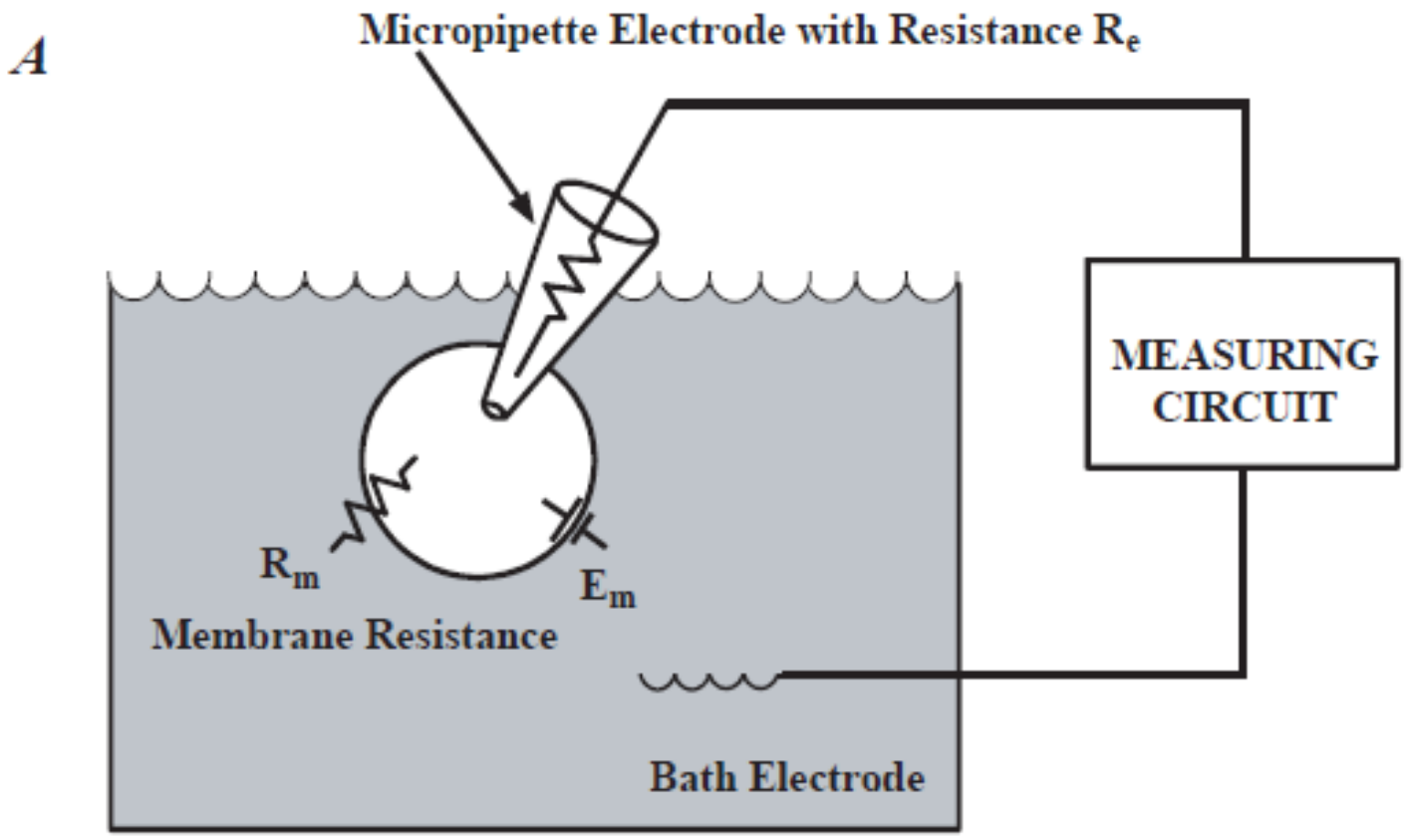
Perforated patch(whole-cell recording)



Nystatin amphotericin B

These drugs create holes in membranes of a very distinct size, permeable to ions but not larger molecules, most critically second messenger molecules such as cAMP.

不同模式下的电路简化图



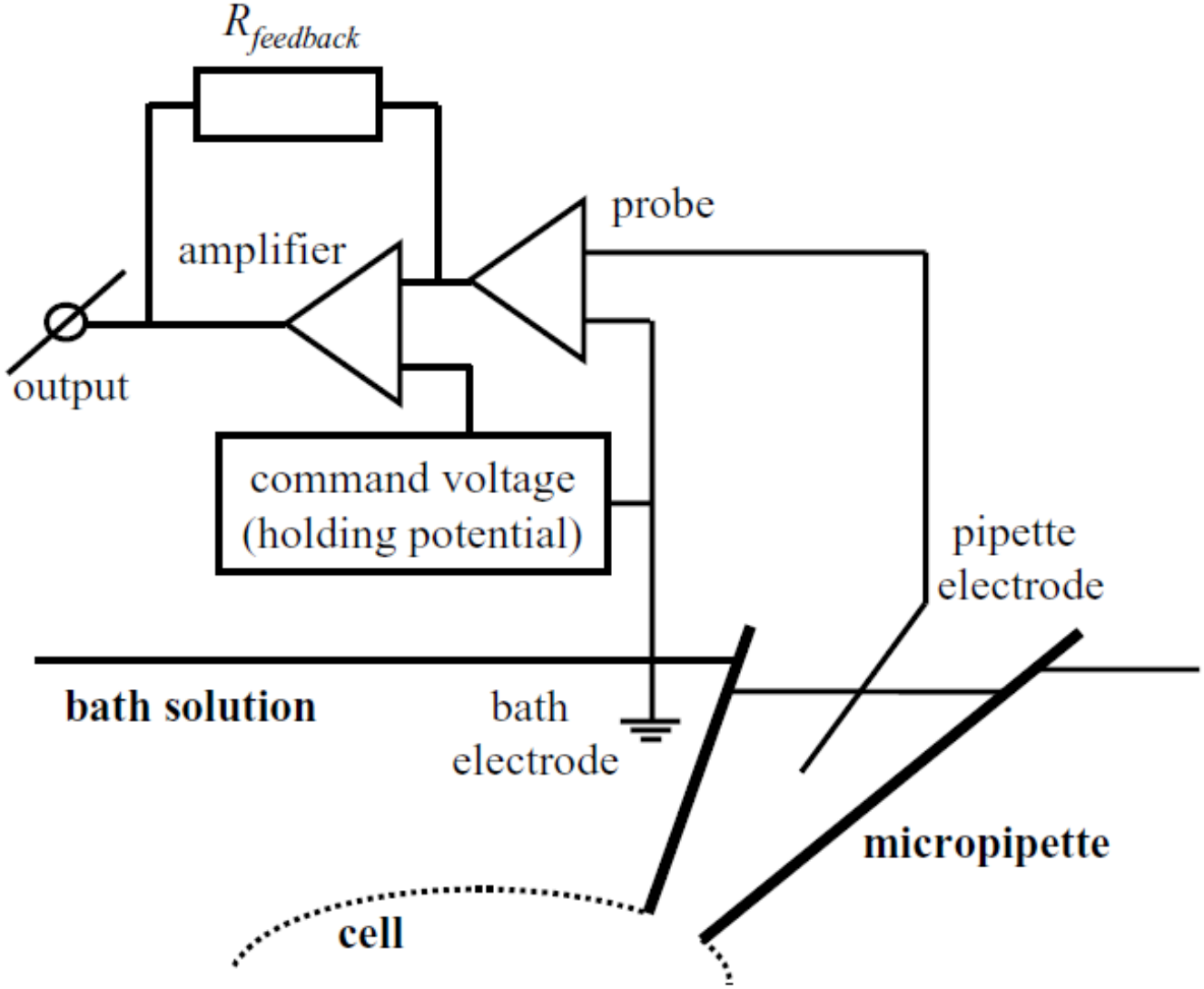


Figure 2.19 The voltage clamp principle in the whole-cell patch configuration.

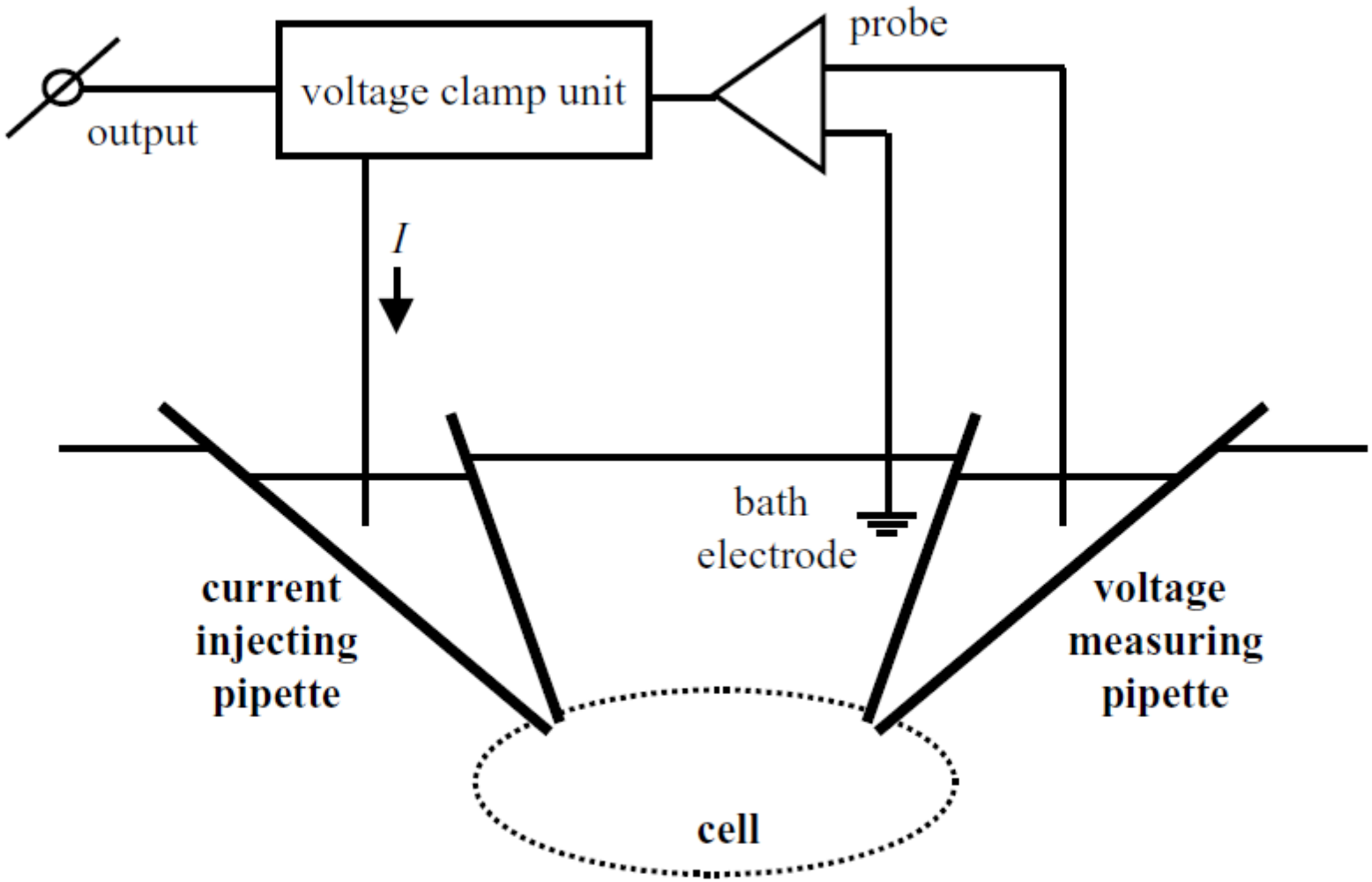


Figure 2.20 The dual-electrode voltage clamp principle

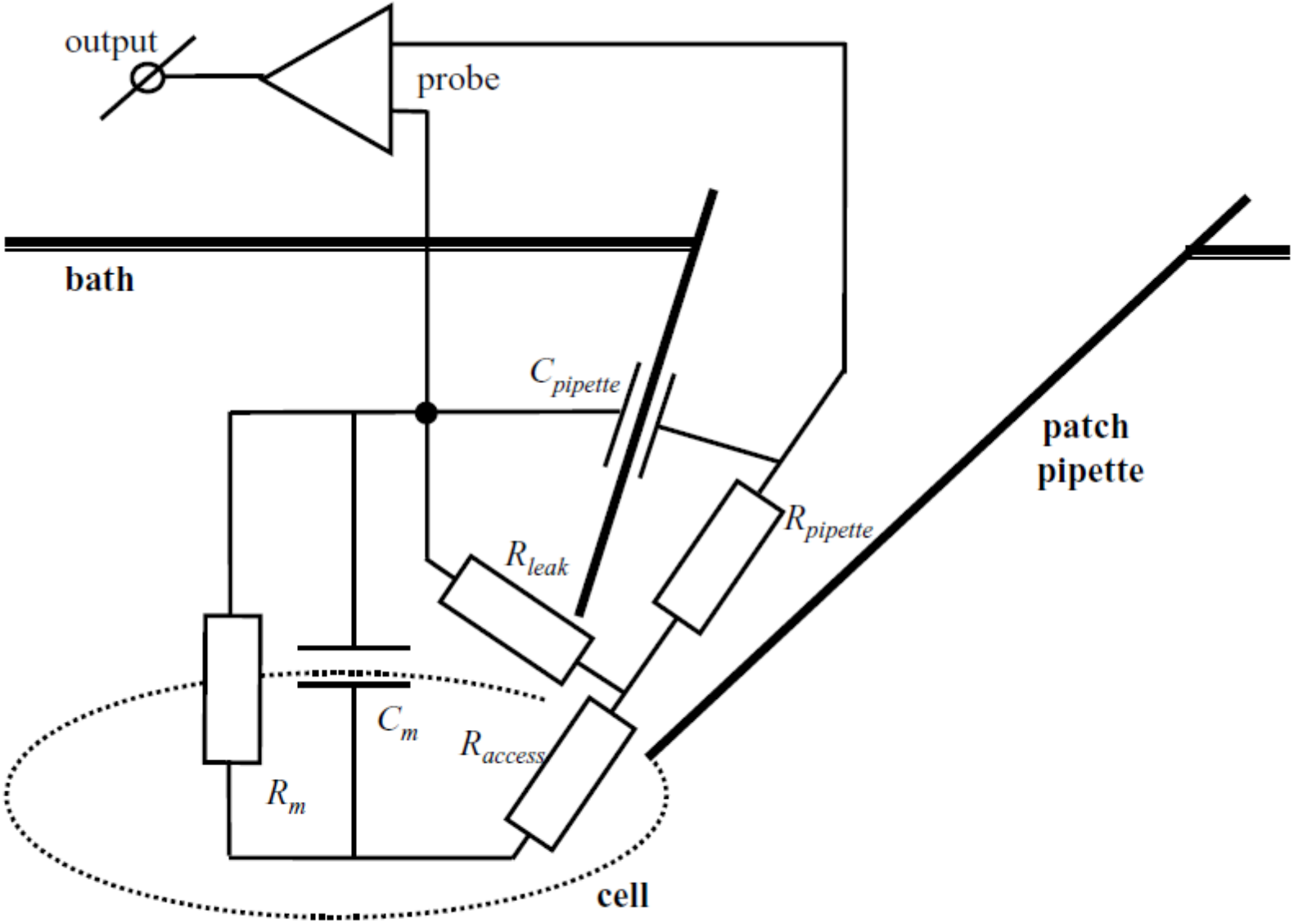


Figure 2.26 Equivalent circuit for the whole-cell configuration

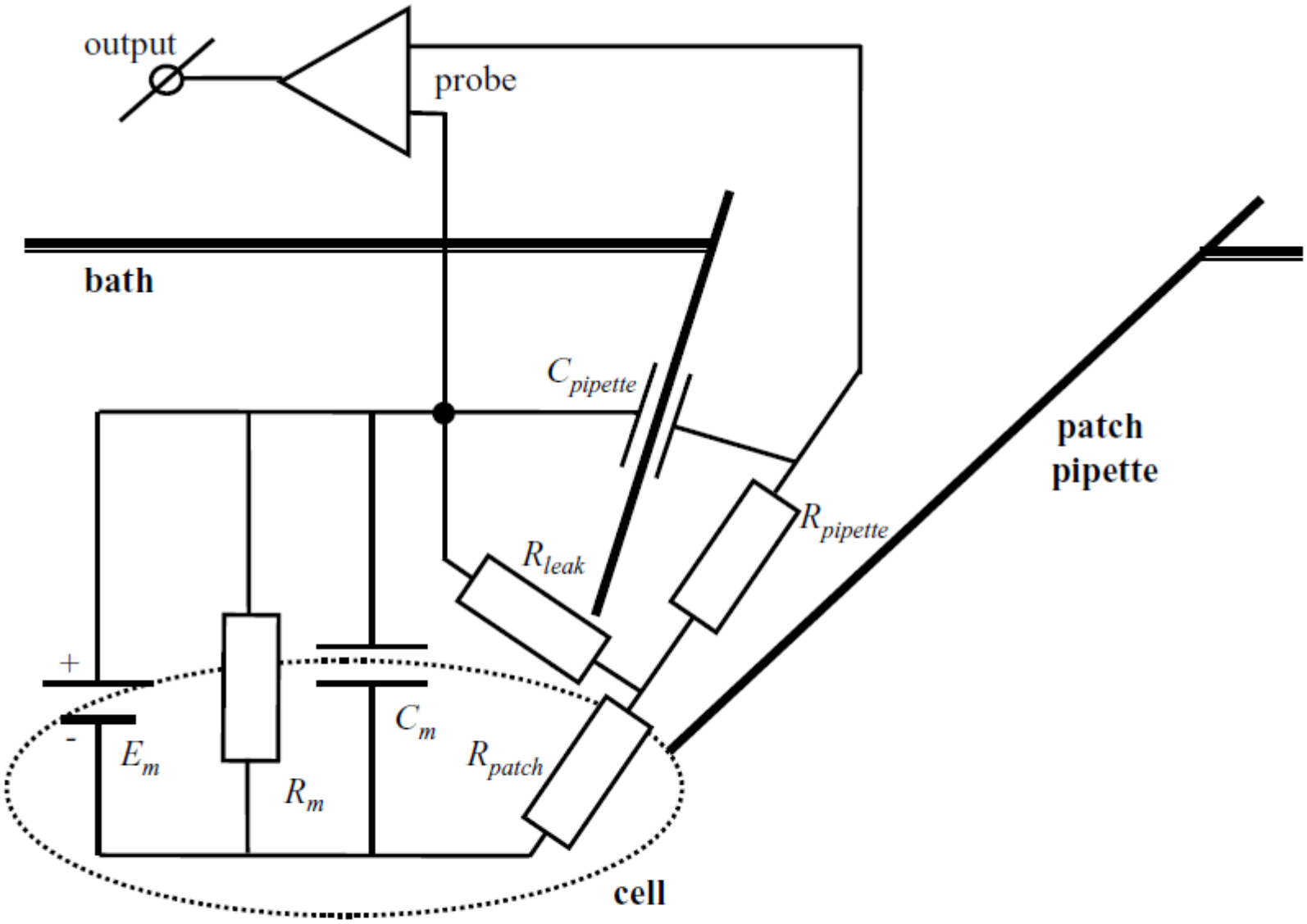


Figure 2.24 Equivalent circuit for the cell-attached patch configuration

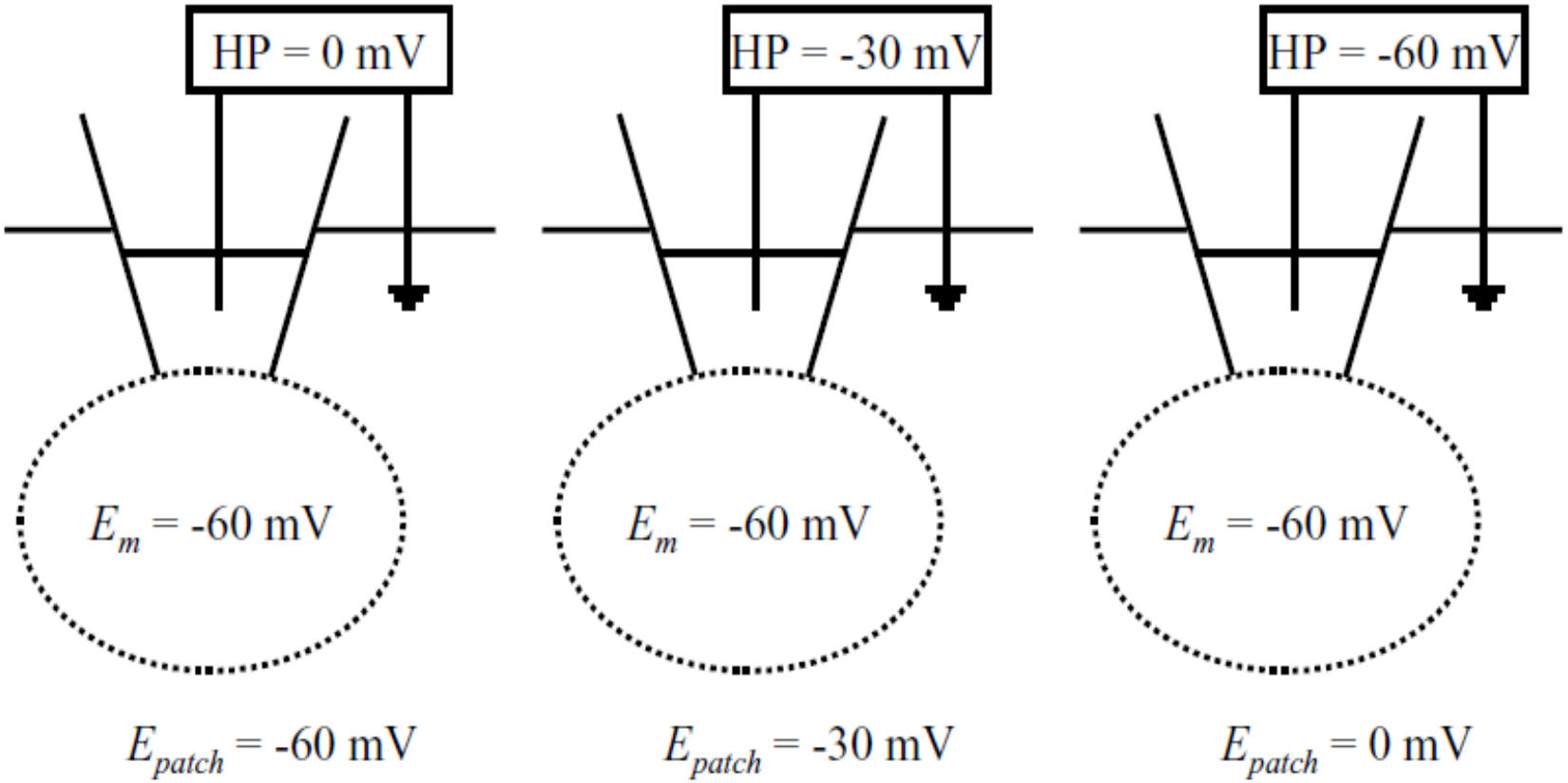


Figure 2.25 Voltage clamp in the cell-attached patch configuration. To obtain the actual potential over the patch, the command voltage (holding potential, HP) must be subtracted from the cell membrane potential

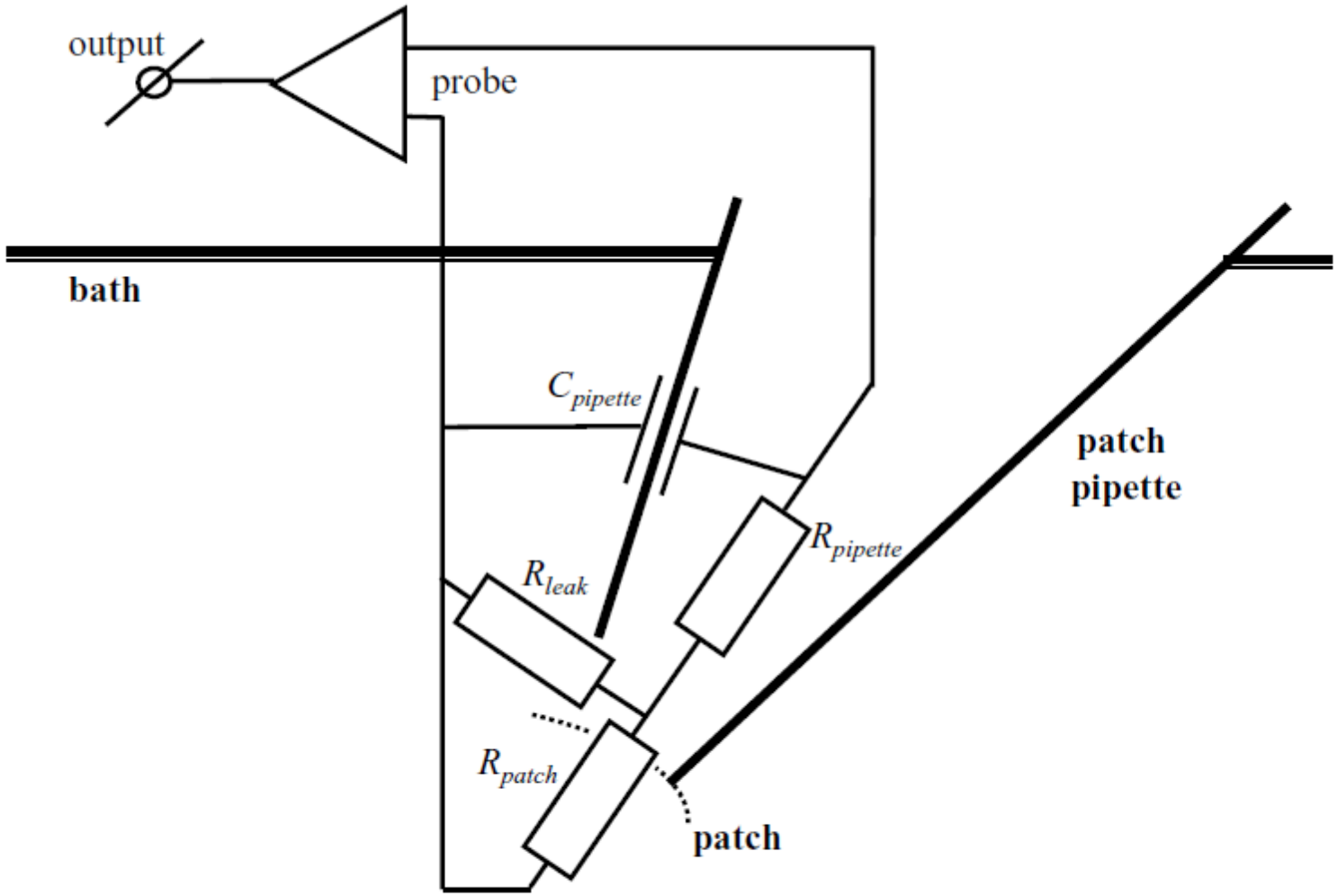


Figure 2.27 Equivalent circuit for excised patch configurations

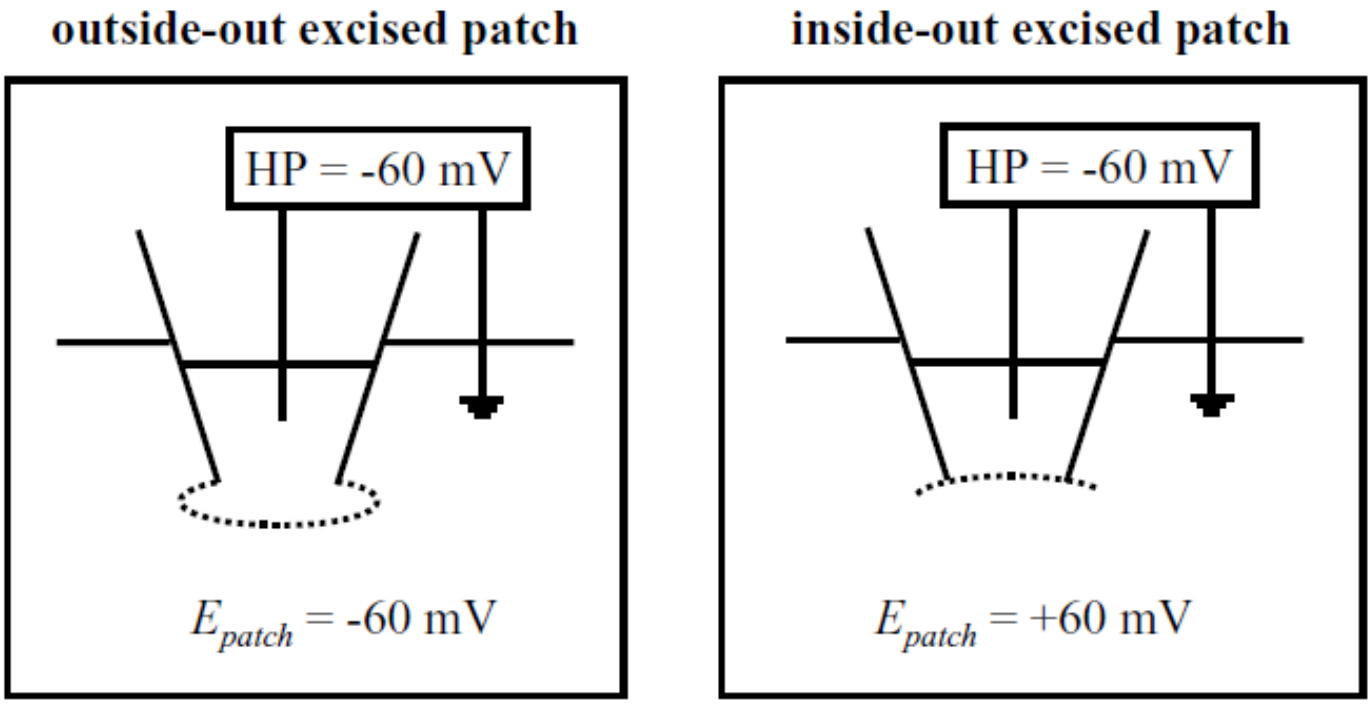


Figure 2.28 Translation of holding potentials (HP) to patch potentials in excised patch configurations

Table 2.3 Summary of advantages and disadvantages of patch clamp configurations

Configuration	Use	Advantages	Disadvantages
Cell-attached patch	Single-channel recording	Cytosolic side intact (physiological) Easy to obtain	Exact patch potential unknown No easy superfusion possible
Outside-out excised patch	Single-channel recording	Extracellular side can be superfused Cytosolic environment is controlled	Washout of cytosolic factors Disruption of cytoskeletal structure
Inside-out excised patch	Single-channel recording	Cytosolic side can be superfused Extracellular environment is controlled	Bath solution must be replaced by intracellular solution Relatively difficult to obtain Disruption of cytoskeletal structure
Whole-cell	Macro-current recording	Quick assertion of ion channel populations Cytosolic environment is controlled Extracellular side can be superfused	Washout of cytosolic factors
Perforated patch	Macro-current recording	Cytosolic ionic environment is controlled No washout of organic factors Extracellular side can be superfused	No control over organic cytosolic factors Relatively difficult to obtain

膜片钳记录流程

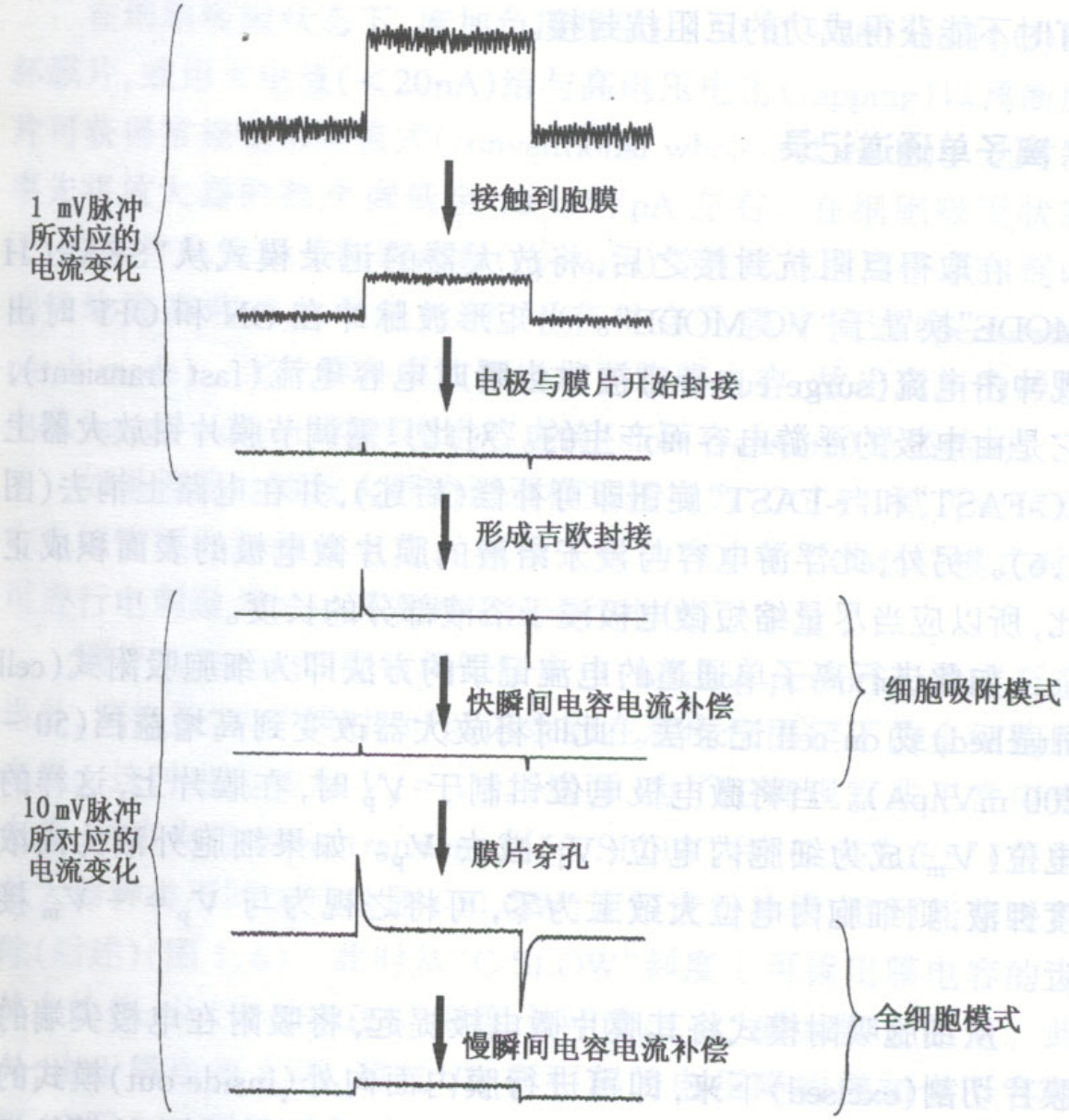
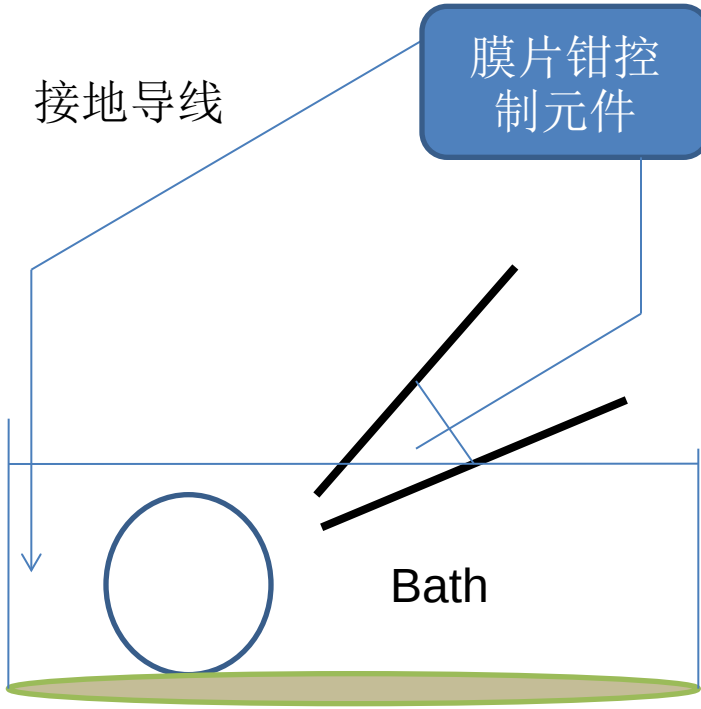


图 1.6　用示波器监视电极阻抗、封接程度和瞬时电容电流补偿的情况。



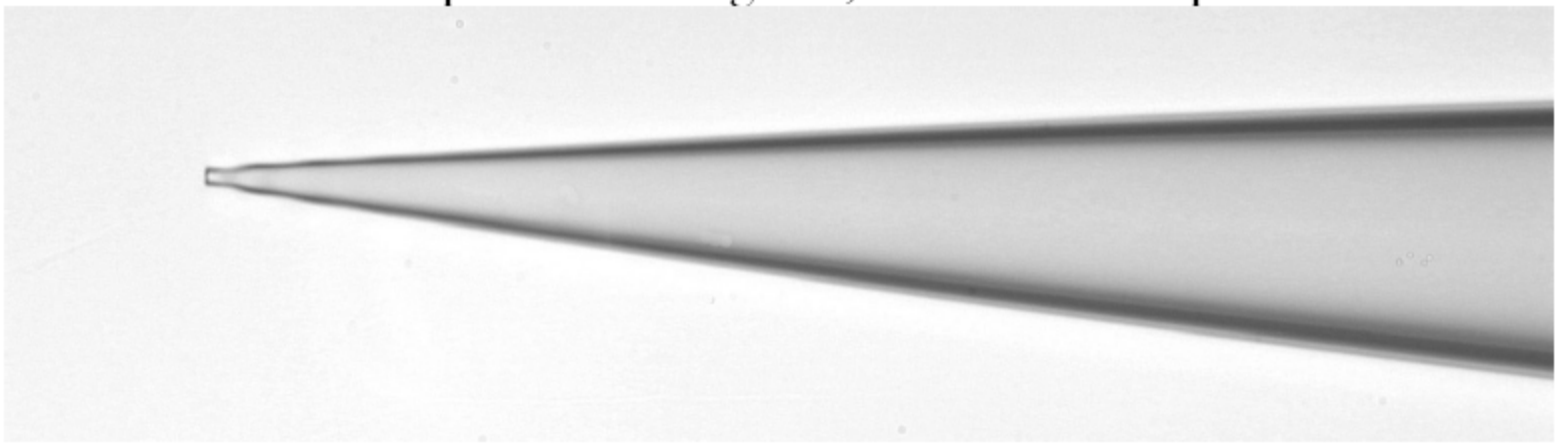


常见问题

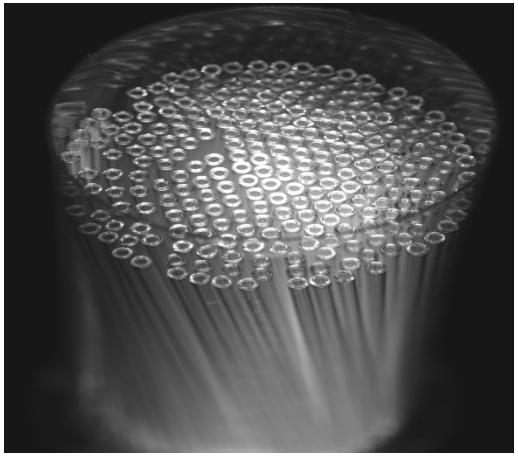
问题1：电极要不要fire polish；sylgard 包被；

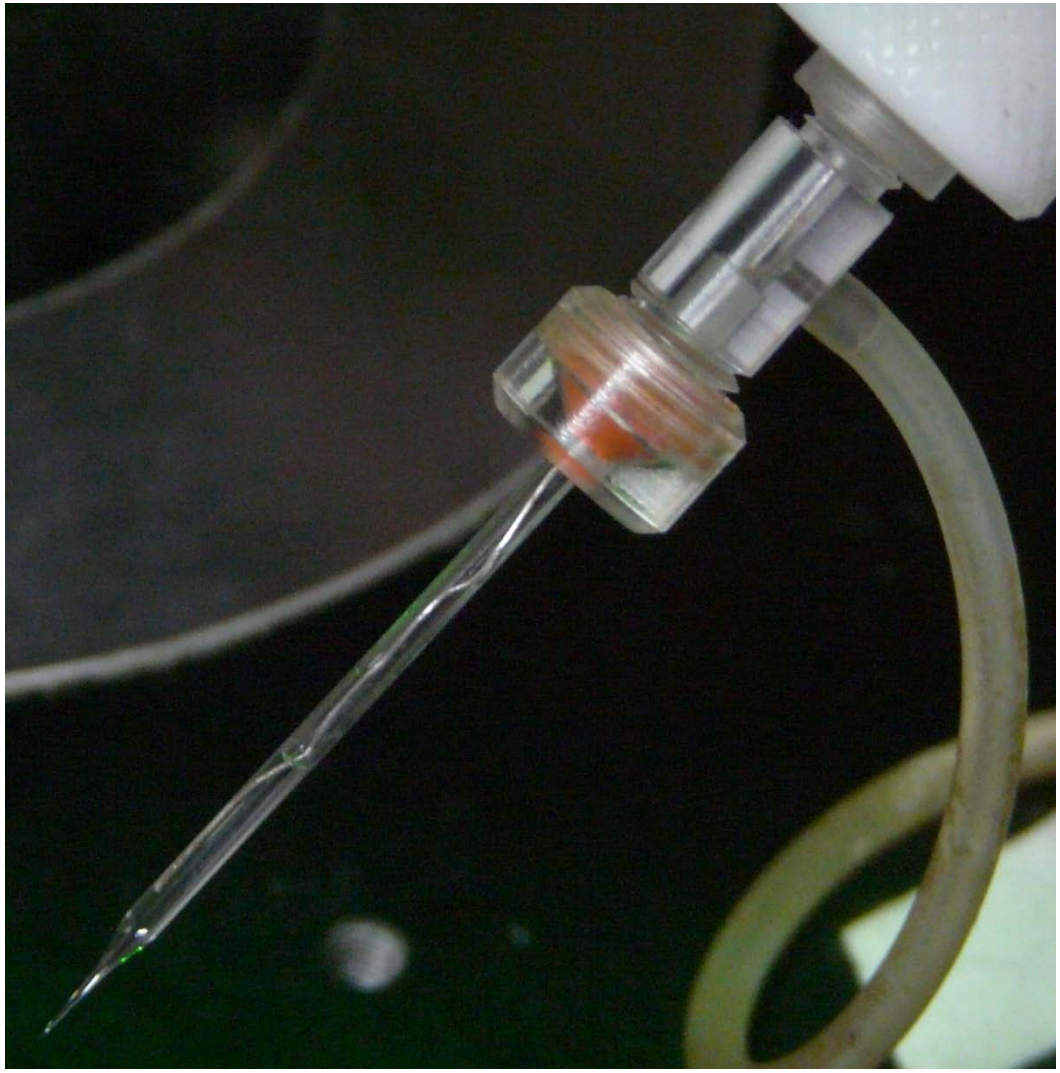
一般情况不需fire polish
遇到难patch细胞可尝试fire polish或用厚壁玻璃电极

Thin Walled Glass (400X mag)
1.5mm x 1.1mm glass, 3.5µm Tip, 4mm taper
▼ General Look Up Tables - Prog #56, two to three loops

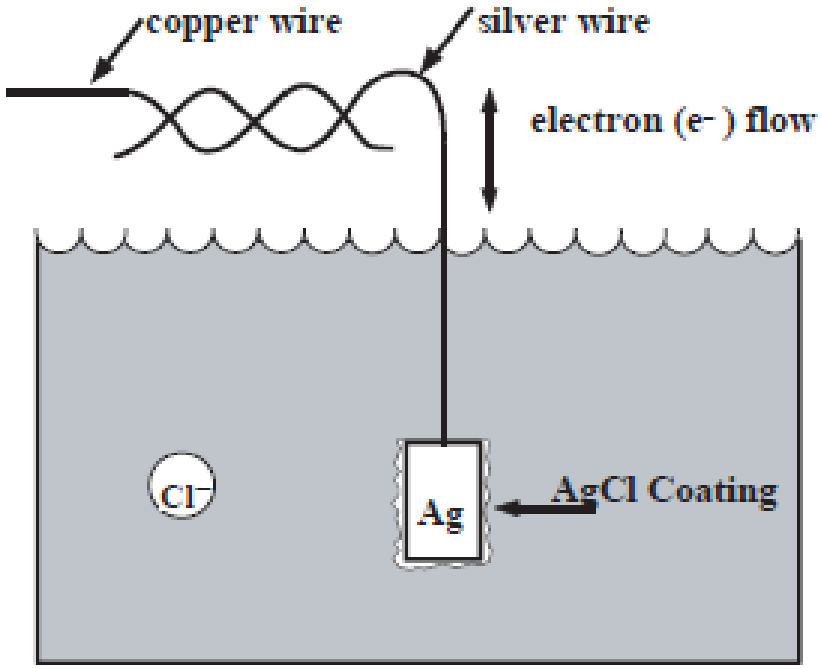


Fire polish ends 防止刮破AgCl





银丝：84消毒液



Electrode reaction:

$$Ag + Cl^- \rightleftharpoons AgCl + \text{electron } (e^-)$$

This reaction can also be presented by:

$$AgCl \rightleftharpoons Ag^+ + Cl^-$$

$$+e^- \quad -e^-$$

$$Ag$$

Figure 1.9: The silver/silver chloride electrode.

The silver/silver chloride electrode is reversible but exhaustible.

sylgard 包被:单通道记录必备

减少电极电容

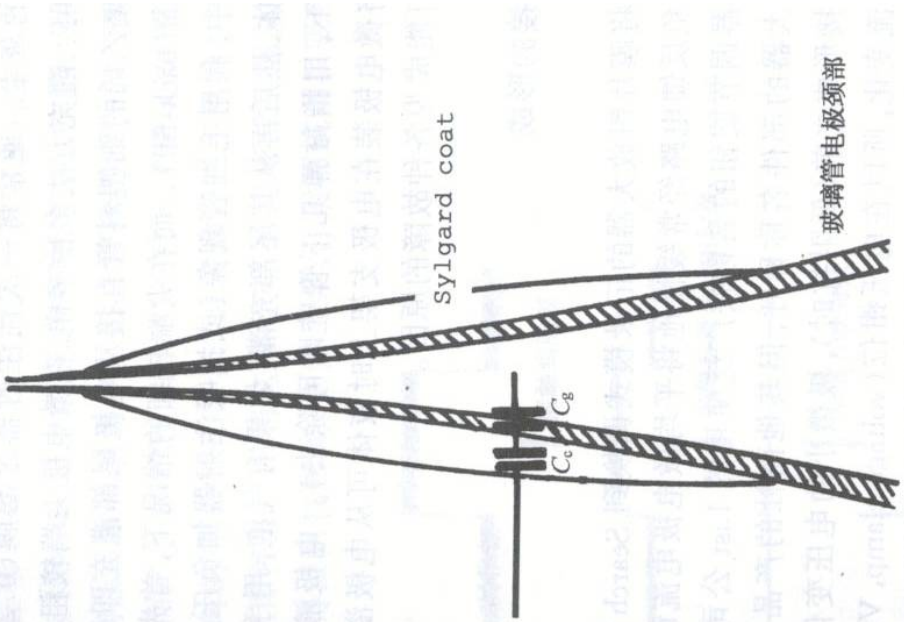


图 1.5 涂硅酮树酯可有效的减小浮游电容。
$C_s = C_c C_g/(C_c + C_g)$如 $C_g >> C_c$,则 $C_s = C_c$。

问题2：膜电容、电极电容

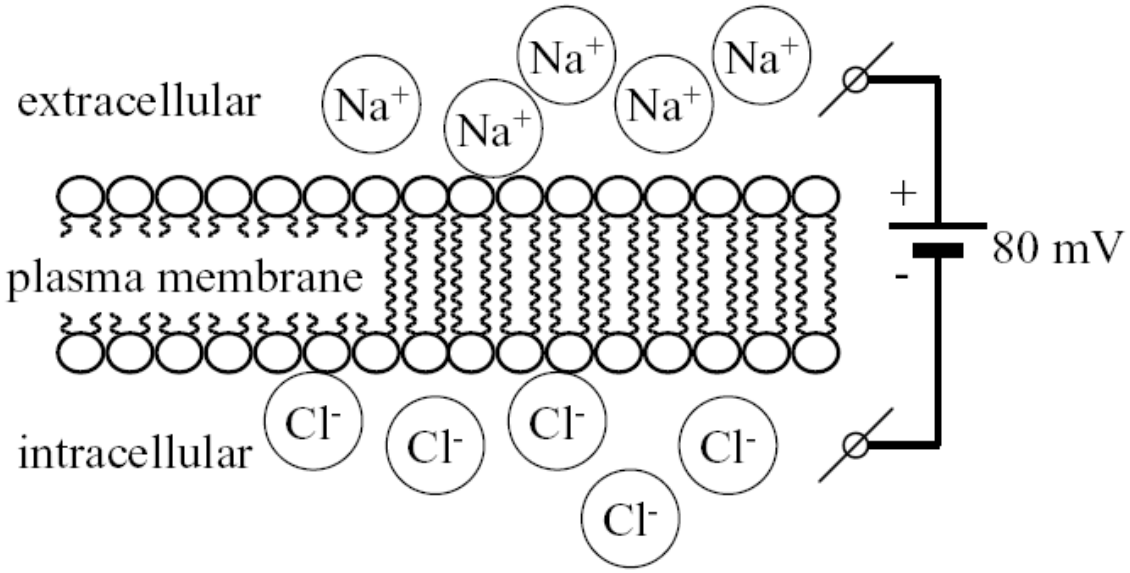


Figure 2.6 The membrane potential charges the membrane like a capacitor

$$Q=EmC$$

Q is the charge stored, Em is the potential difference across the membrane and C is the membrane capacitance (expressed in farad).

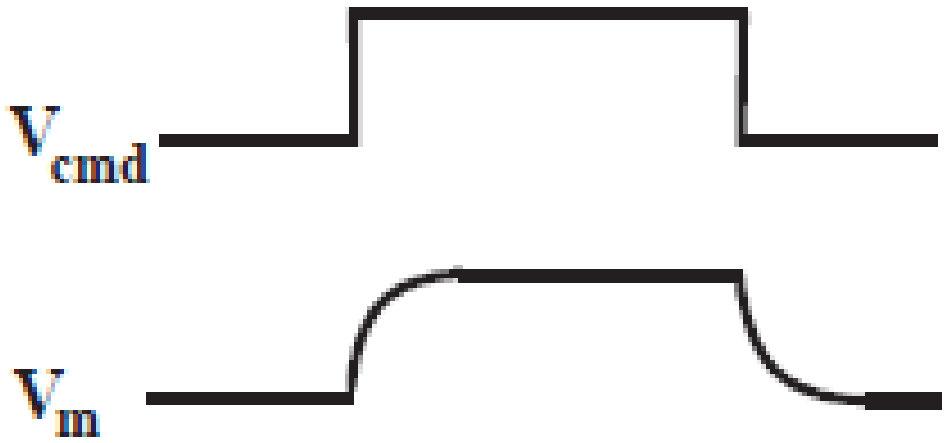
$$C = \frac{A\varepsilon_r}{d}$$

A is the membrane area,εr is the dielectric constant for the membrane and d is the membrane thickness.

细胞的厚度基本一致，所以可以用膜电容来估计细胞的大小。
0.01pF/μm^2

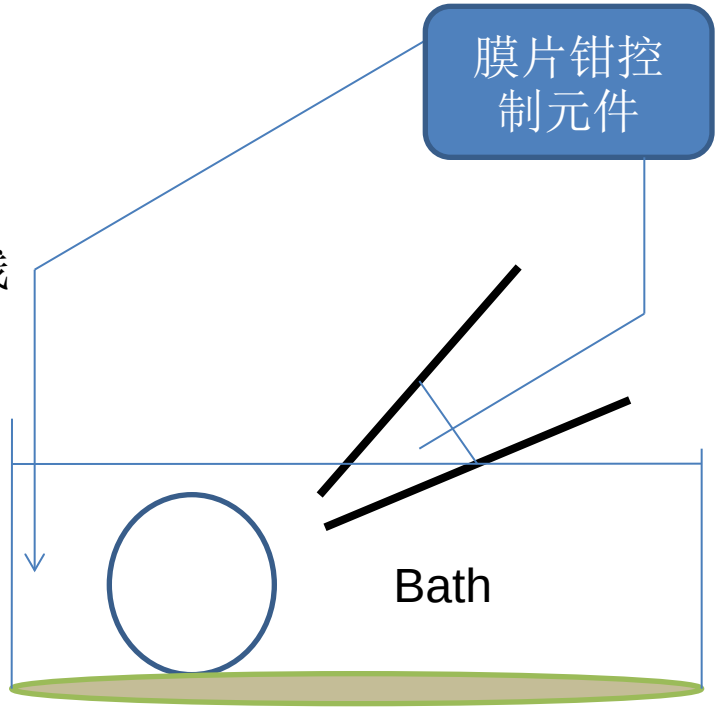
全细胞电流的大小常用电流的密度表示：电流/电容

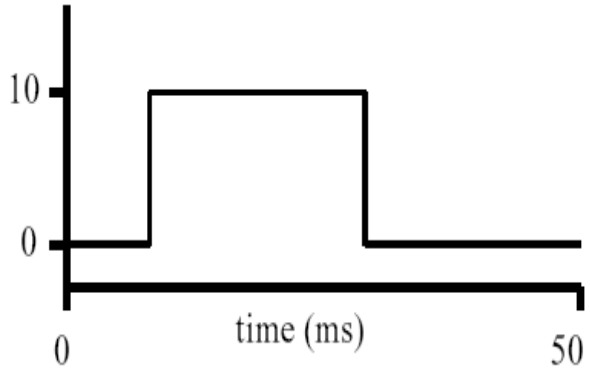
膜电容的影响



问题2：调零

一切干扰电流（电极未和细胞接触）都被去除，但错杀了Liquid junction potentials



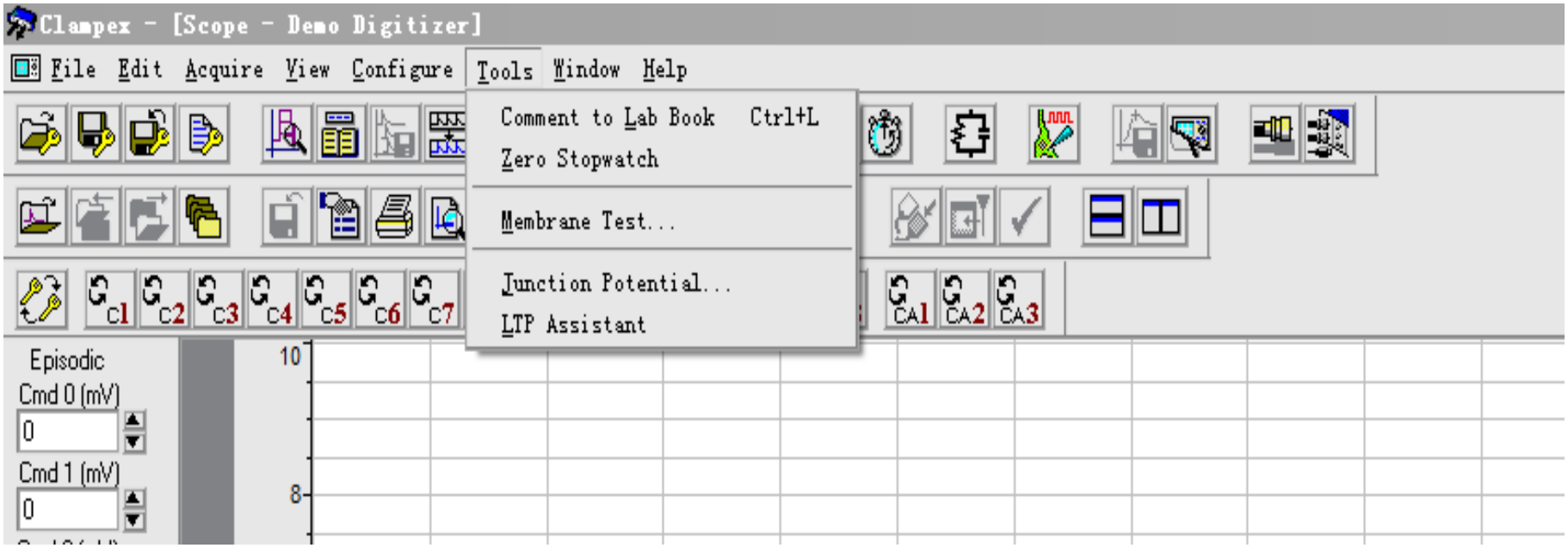


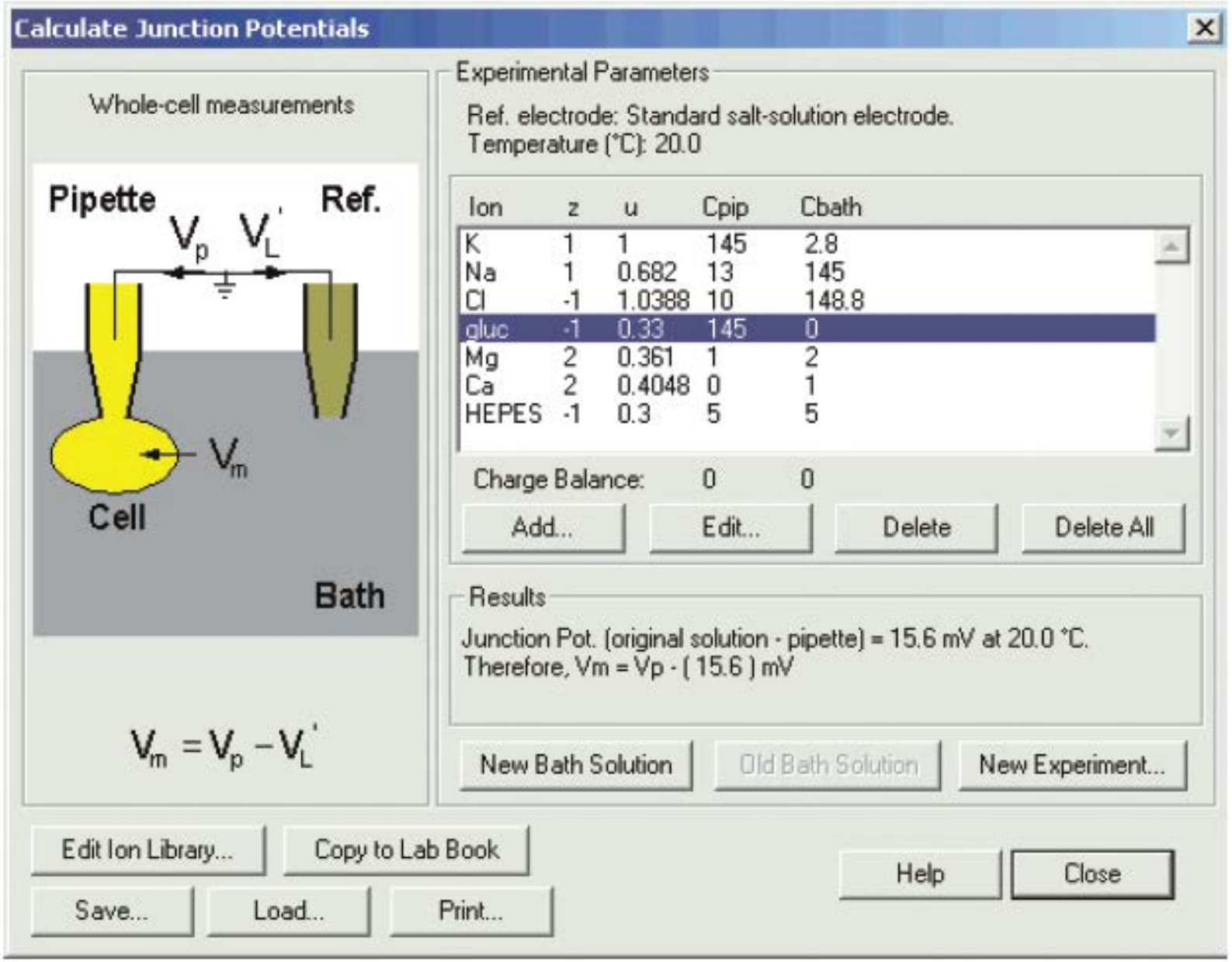
Liquid junction potentials

两种不同的盐溶液在接触面产生的电位差：
离子浓度不同；离子的迁移率不同

计算LJP：Axon pclamp 软件

Tools/junction potential





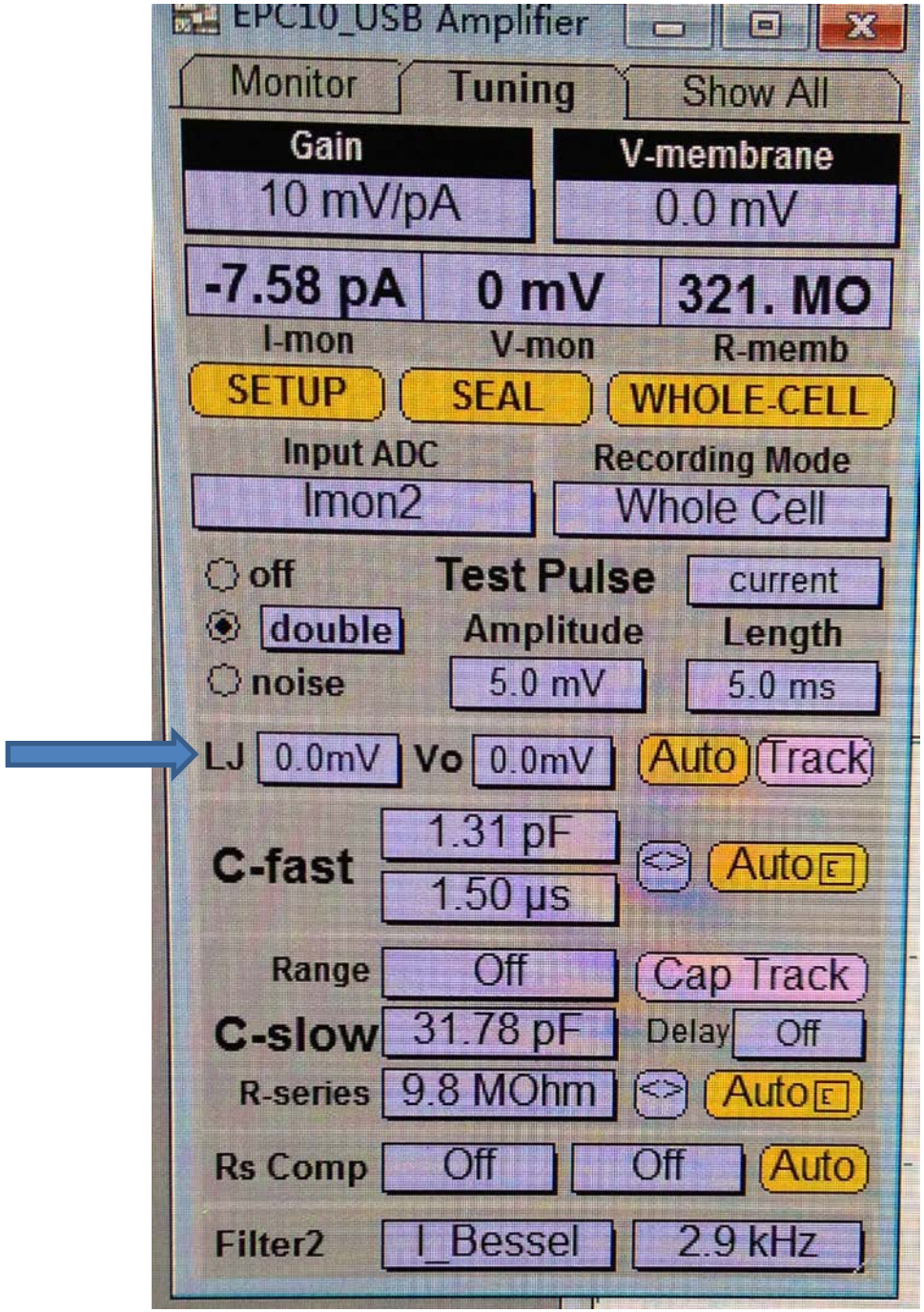
注意：HEPES 10mM ,通常pH后产生5mM HEPES- 和5mM 阳离子

Whole cell mode :

电压钳：
Vm=Vcmd-LJP

电流钳：
Vm=Vrec-LJP

单通道记录根据内外液的方向确定最后的计算方法。
Outside out is same with whole cell mode.
Inside out :Vm=-Vcmd+LJP


EPC10_USB Amplifier
Monitor
Tuning
Show All
Gain
10 mV/pA
V-membrane
0.0 mV
-7.58 pA
0 mV
321. MO
I-mon
V-mon
R-memb
SETUP
SEAL
WHOLE-CELL
Input ADC
Imon2
Recording Mode
Whole Cell
off
Test Pulse
current
double
Amplitude
Length
noise
5.0 mV
5.0 ms
LJ
0.0mV
Vo
0.0mV
Auto
Track
C-fast
1.31 pF
1.50 µs
Auto
Range
Off
Cap Track
C-slow
31.78 pF
Delay
Off
R-series
9.8 MOhm
Auto
Rs Comp
Off
Off
Auto
Filter2
I_Bessel
2.9 kHz

问题3：补偿

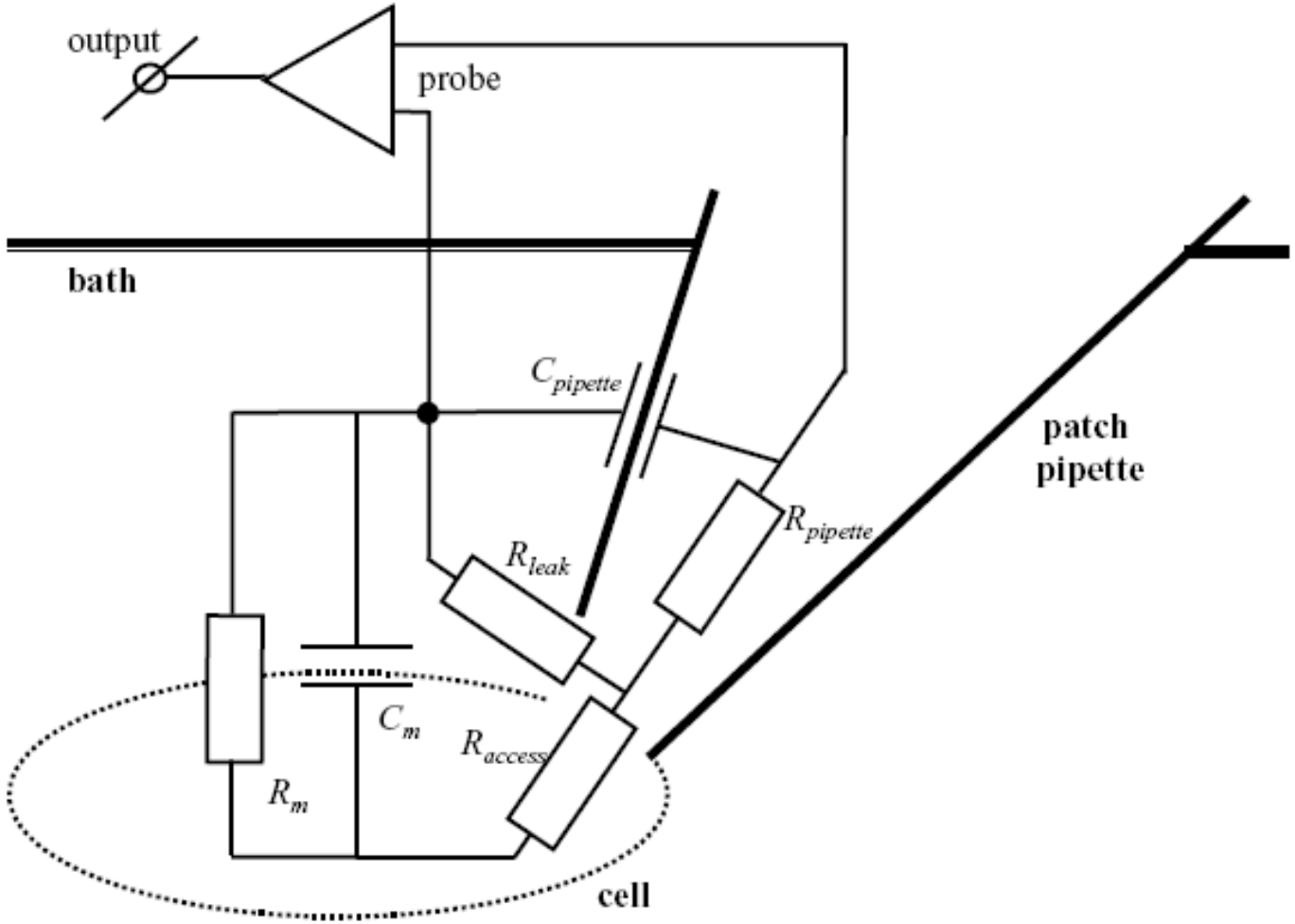
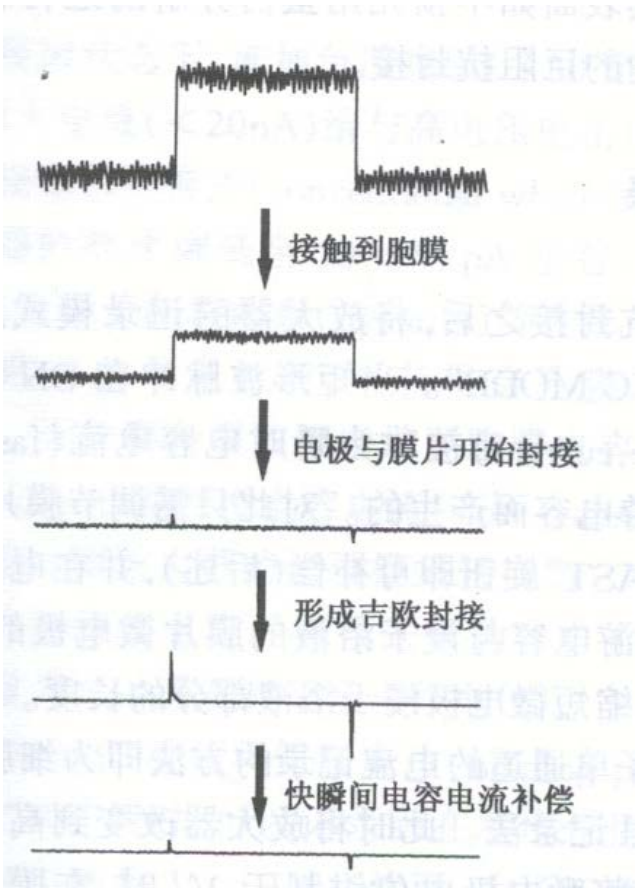


Figure 2.26 Equivalent circuit for the whole-cell configuration

1、补偿电极电容





Pipette capacitance compensation

Fast MAG





MultiClamp 700B : (00831329)

1 2 3 ?

Channel 1

V (mV) -91.9

I (pA) 3.4

Resistance Irms

Mode

VC I=0 IC Ext

V-Clamp 1 | I-Clamp 1

Holding: 0 mV

Seal Tes 10 mV @ 100 Hz

Pipette

8.72 mV Auto

Cp 7.137 pF 0.917 衽 Auto

Cp 0.00 pF 10.0 衽 Tau x20 Auto

Whole Cel

10.54 pF 29.32 MΩ

Auto

Rs Compensatior

Bandwidth 1.02 kHz

Correcti 0 % Predicti 0 %

Disable if oscillation

Primary Membrane Current (0.5 V/nA)

Gain:1 Bessel: 1 kHz AC: DC Scope: Bypass

Output Zer

0 mV Auto

Leak Subtracti

10.00 MΩ Auto

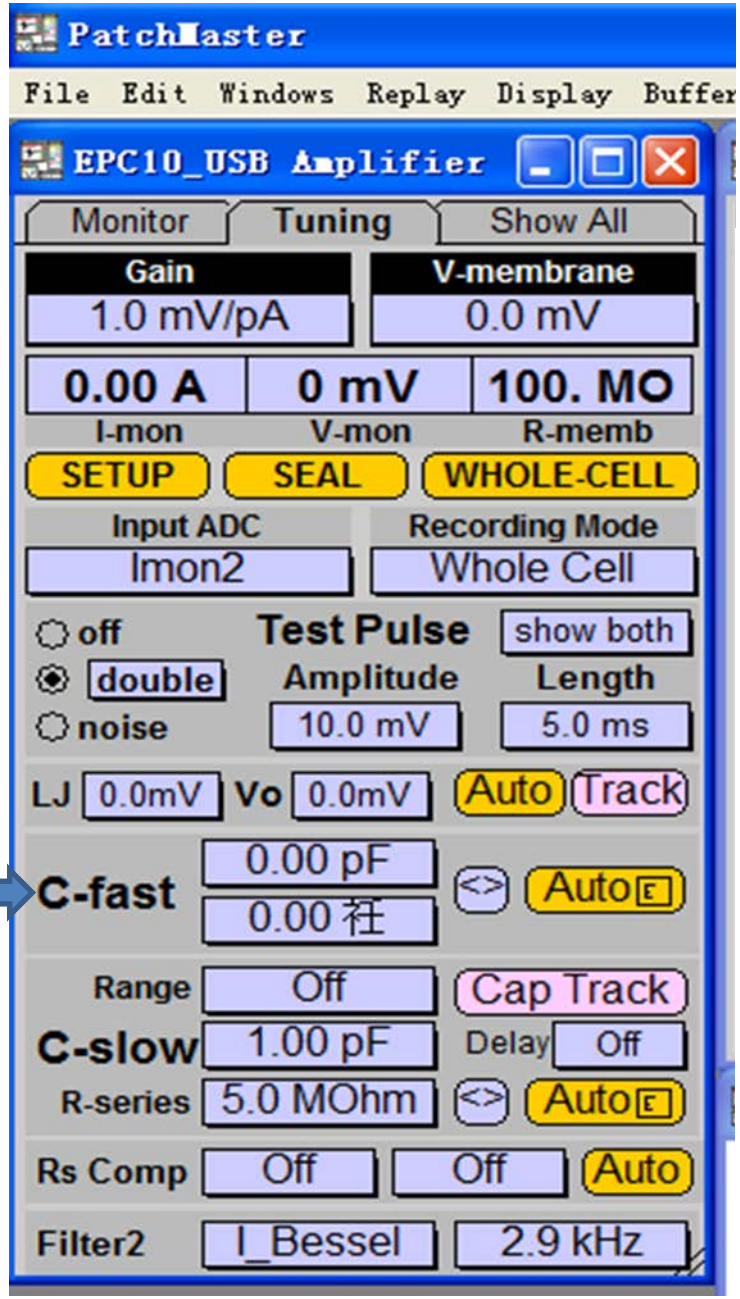
Secondary Membrane Potential (10 mV/mV)

Gain: 1 Lowpass 10 kHz

Pulse 10 mV 10 ms

Zap 500 衽

Rf:500 MΩ



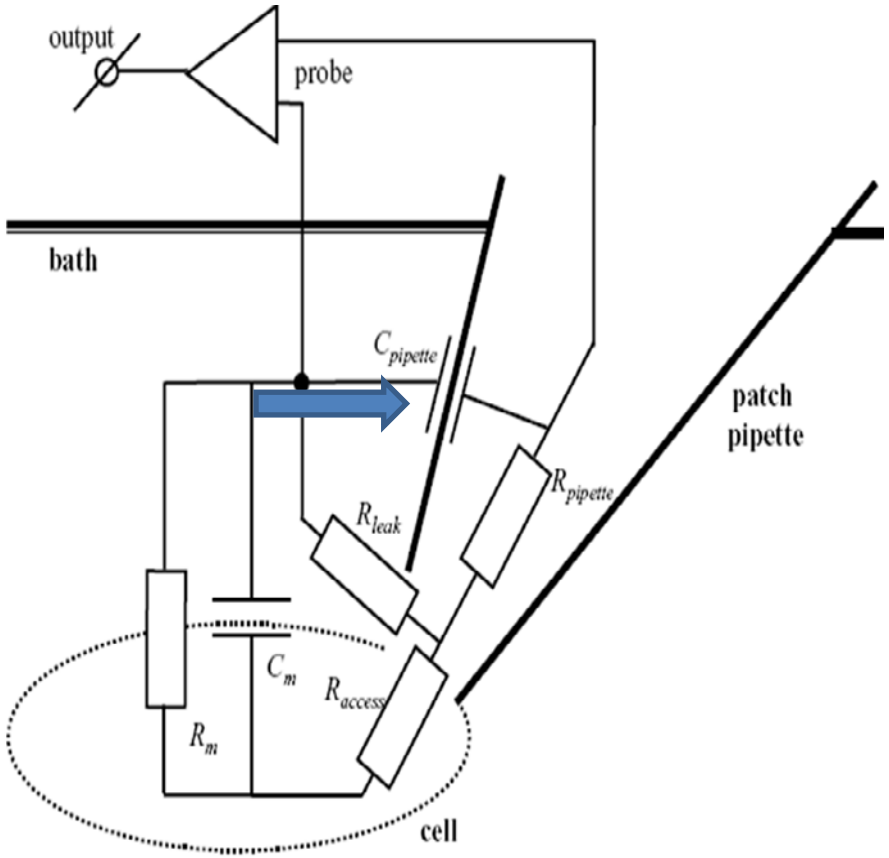
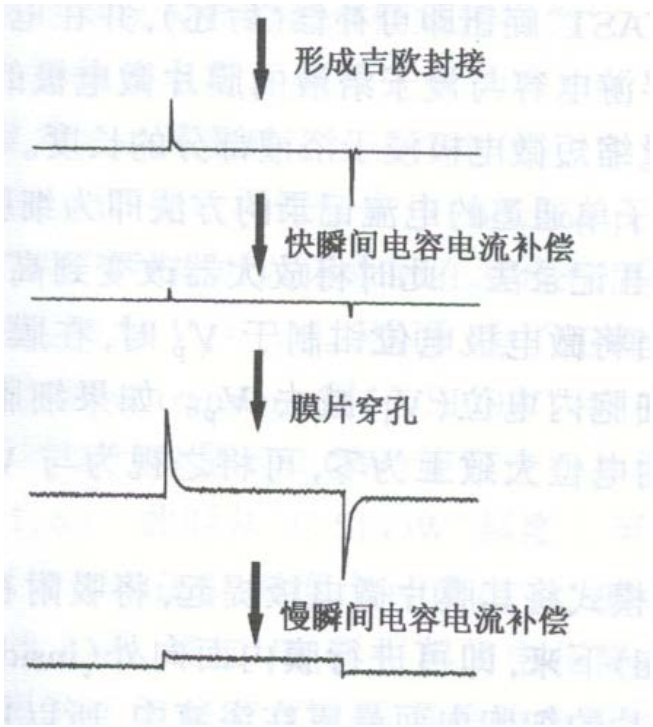


Figure 2.26 Equivalent circuit for the whole-cell configuration

2. 补偿膜电容

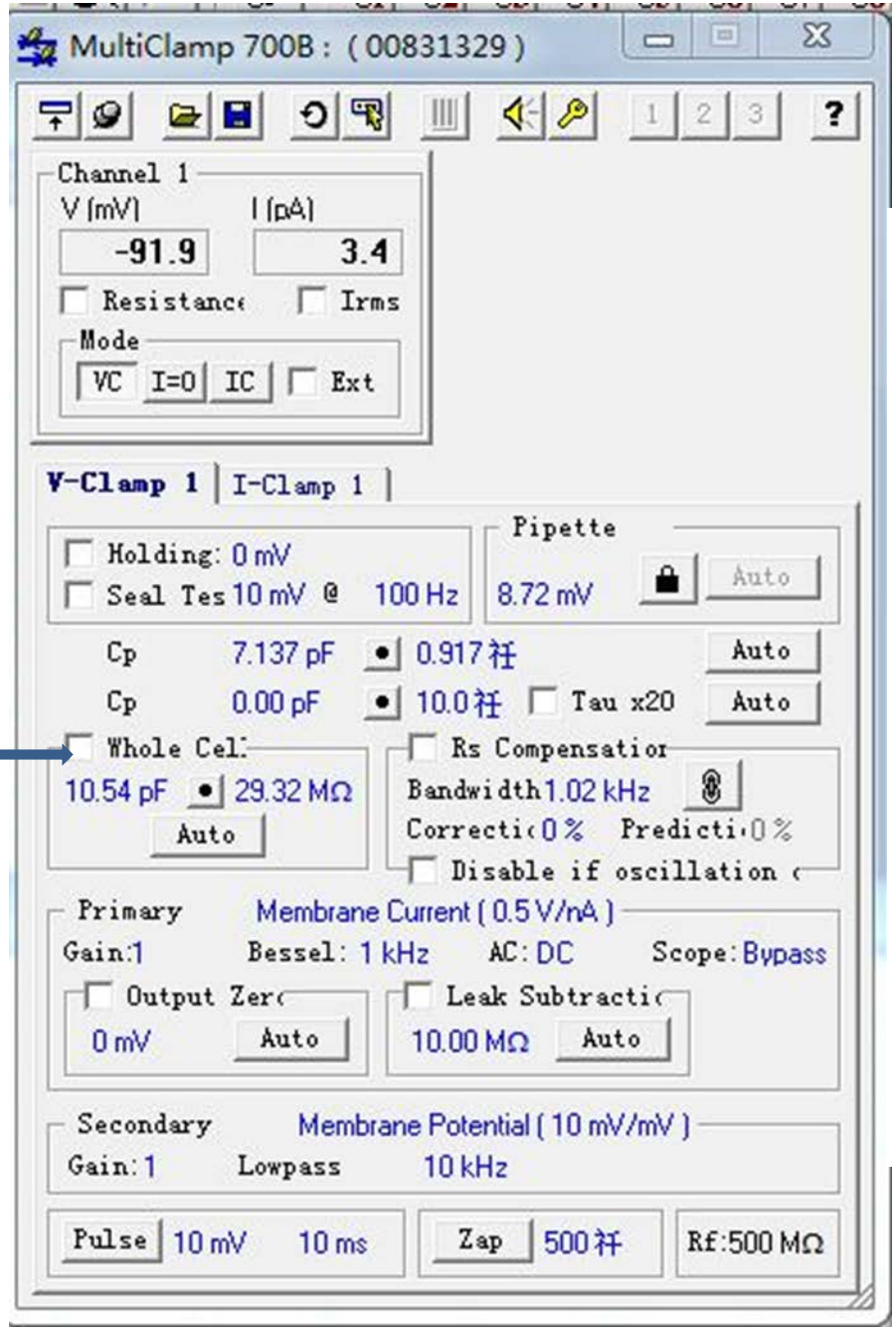


Whole cell cap (Cs)

Series resistance(R_s)=R_p+R_a

R_s<20





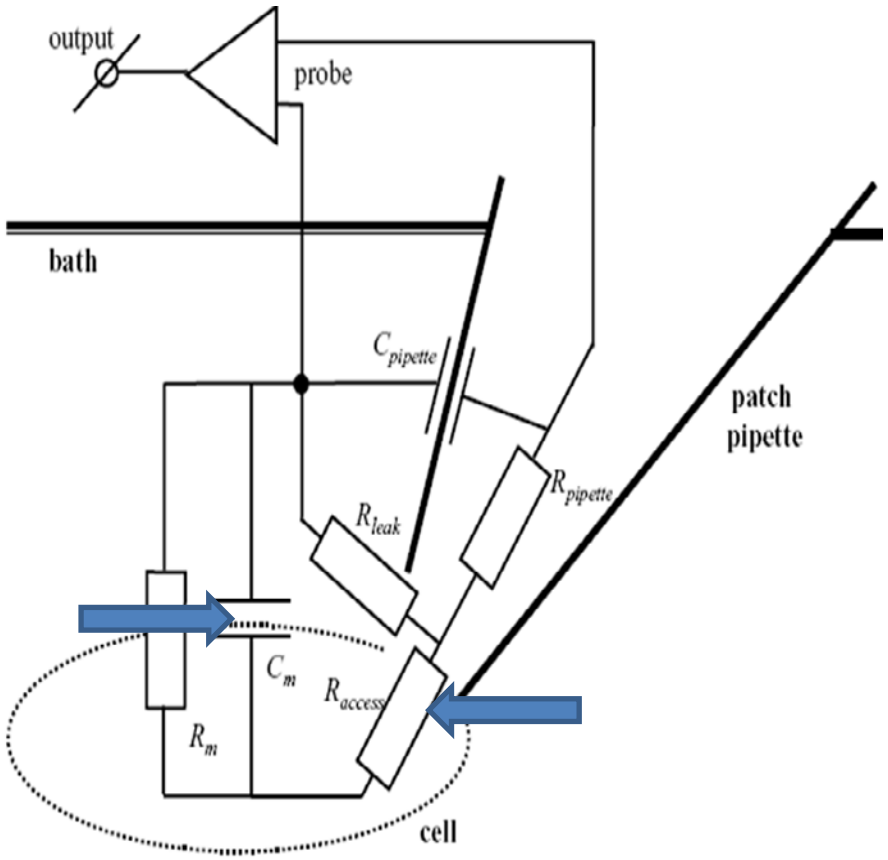
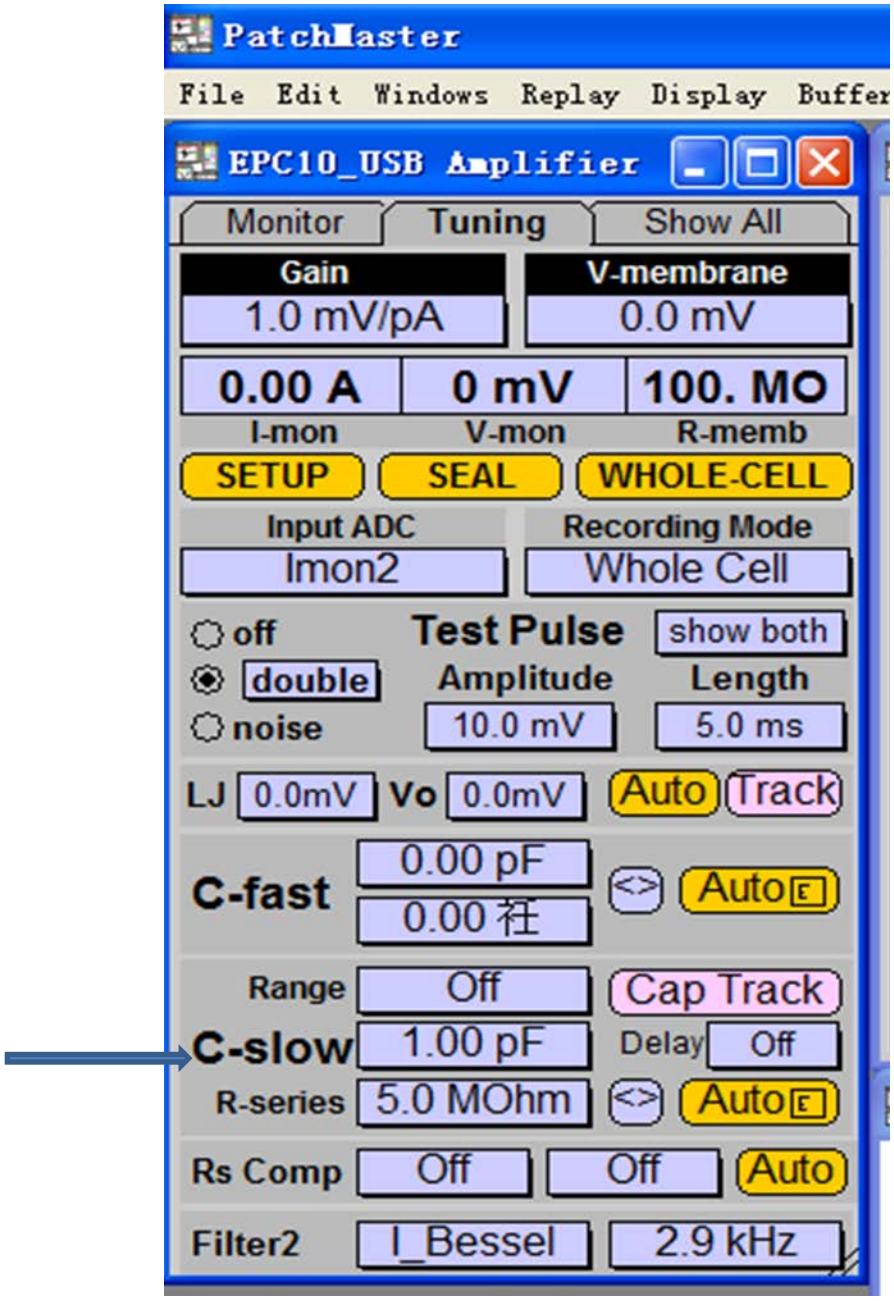
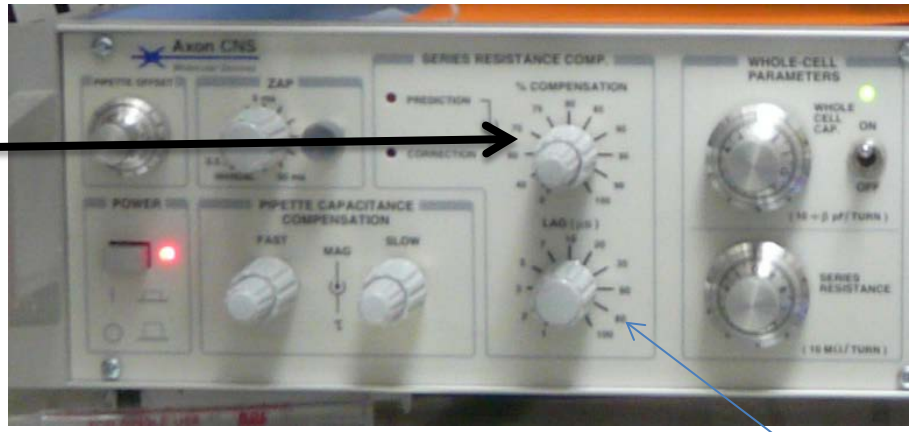


Figure 2.26 Equivalent circuit for the whole-cell configuration


PatchMaster
File Edit Windows Replay Display Buffer
EPC10_USB Amplifier
Monitor
Tuning
Show All
Gain
1.0 mV/pA
V-membrane
0.0 mV
0.00 A
0 mV
100. MO
I-mon
V-mon
R-memb
SETUP
SEAL
WHOLE-CELL
Input ADC
Imon2
Recording Mode
Whole Cell
off
Test Pulse
show both
double
Amplitude
Length
noise
10.0 mV
5.0 ms
LJ
0.0mV
Vo
0.0mV
Auto
Track
C-fast
0.00 pF
0.00 pF
Auto
Range
Off
Cap Track
C-slow
1.00 pF
Delay
Off
R-series
5.0 MOhm
Auto
Rs Comp
Off
Off
Auto
Filter2
I_Bessel
2.9 kHz

3. 补偿串联电阻（R_s）

% compensation



举例：

1nA*20MΩ=20mV

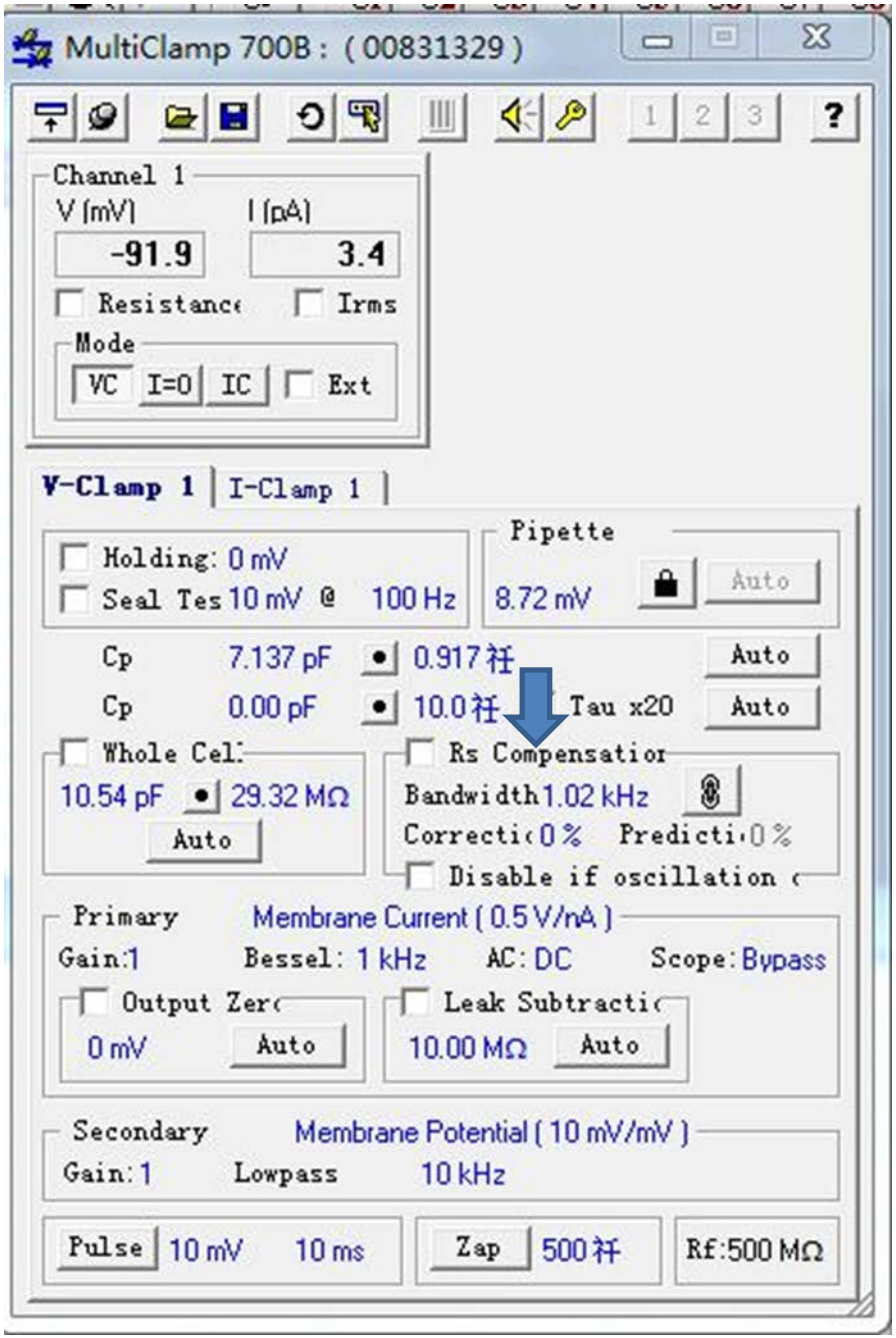
误差20mV, 若补偿90%，则误差为10%*20=2mV

补偿越大越好但系统负担会过大，一般补偿60或70%

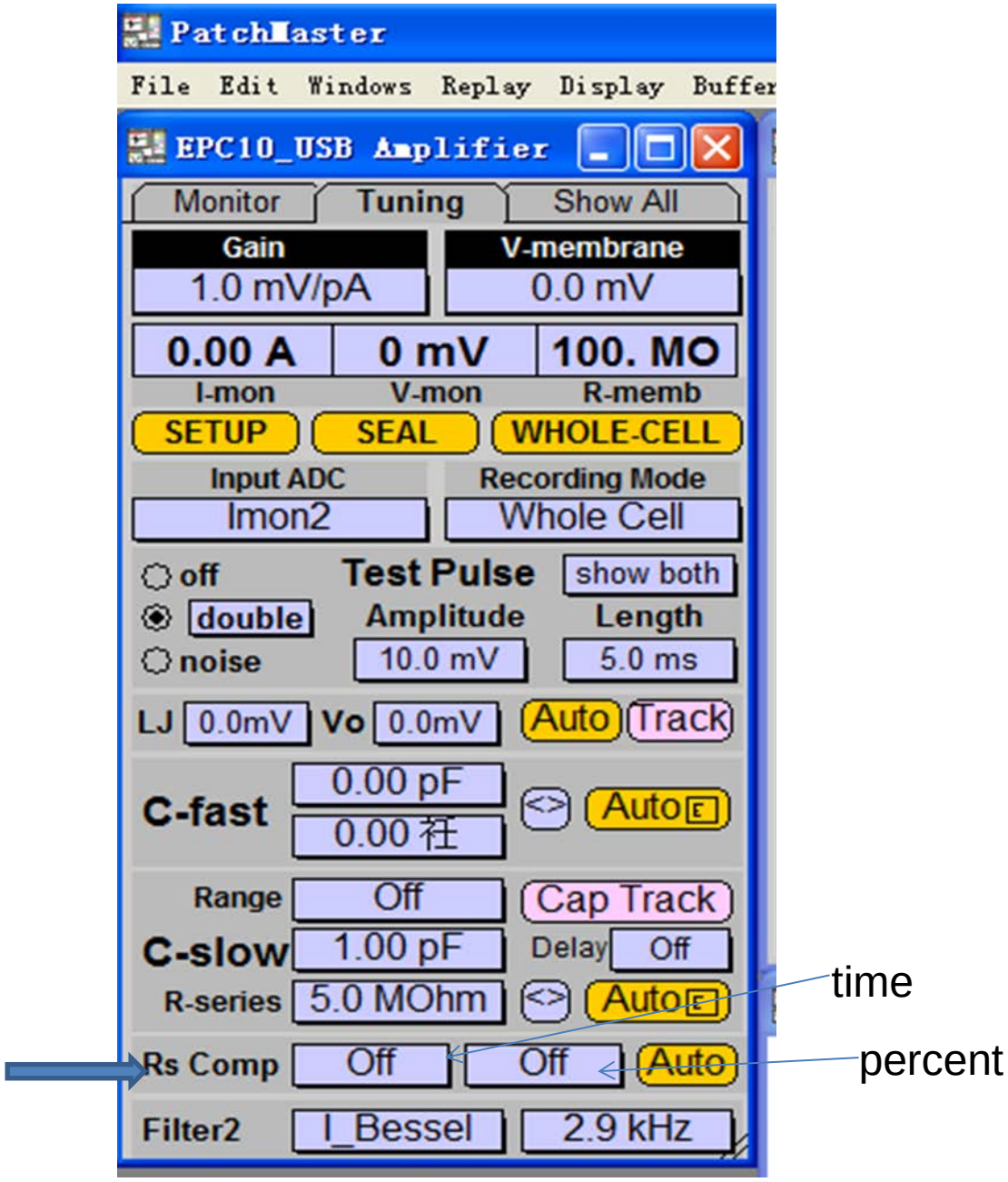
LAG调到10υs左右

(10-20)

10υs=16kHz
20υs=8kHz



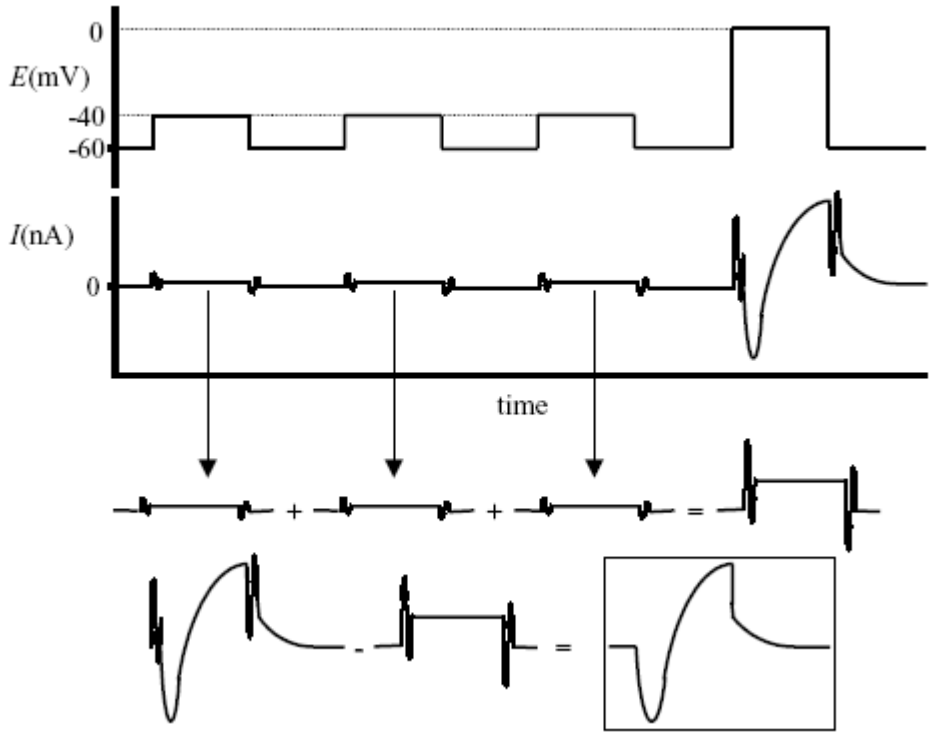
10υs=16kHz
20υs=8kHz


PatchMaster
File Edit Windows Replay Display Buffer
EPC10_USB Amplifier
Monitor
Tuning
Show All
Gain
1.0 mV/pA
V-membrane
0.0 mV
0.00 A
0 mV
100. MO
I-mon
V-mon
R-memb
SETUP
SEAL
WHOLE-CELL
Input ADC
Imon2
Recording Mode
Whole Cell
off
double
noise
Test Pulse
show both
Amplitude
Length
10.0 mV
5.0 ms
LJ
0.0mV
Vo
0.0mV
Auto
Track
C-fast
0.00 pF
0.00 pF
Auto
Range
Off
Cap Track
C-slow
1.00 pF
Delay
Off
R-series
5.0 MOhm
Auto
Rs Comp
Off
Off
Auto
Filter2
I_Bessel
2.9 kHz
time
percent

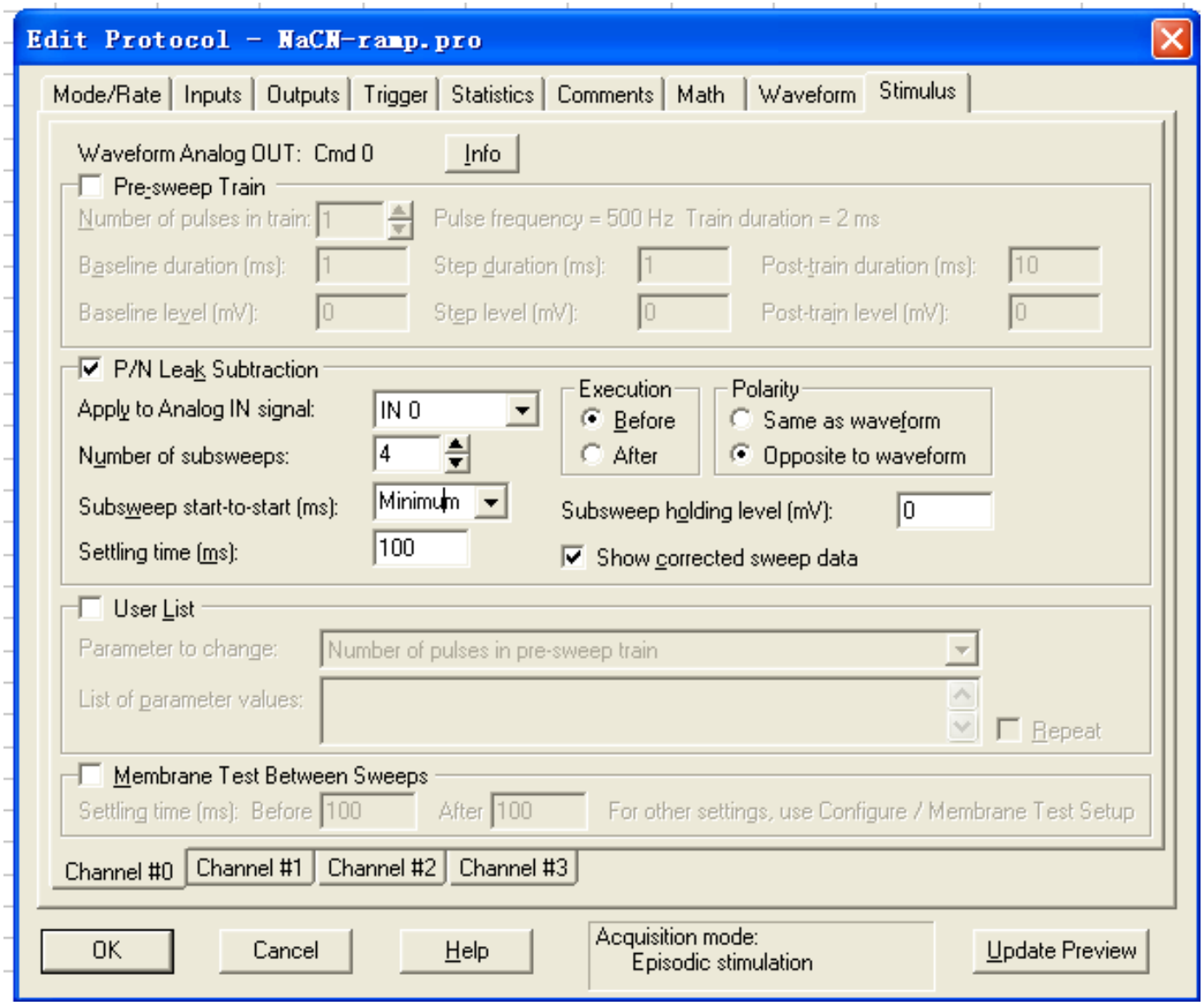
4. 漏电流补偿

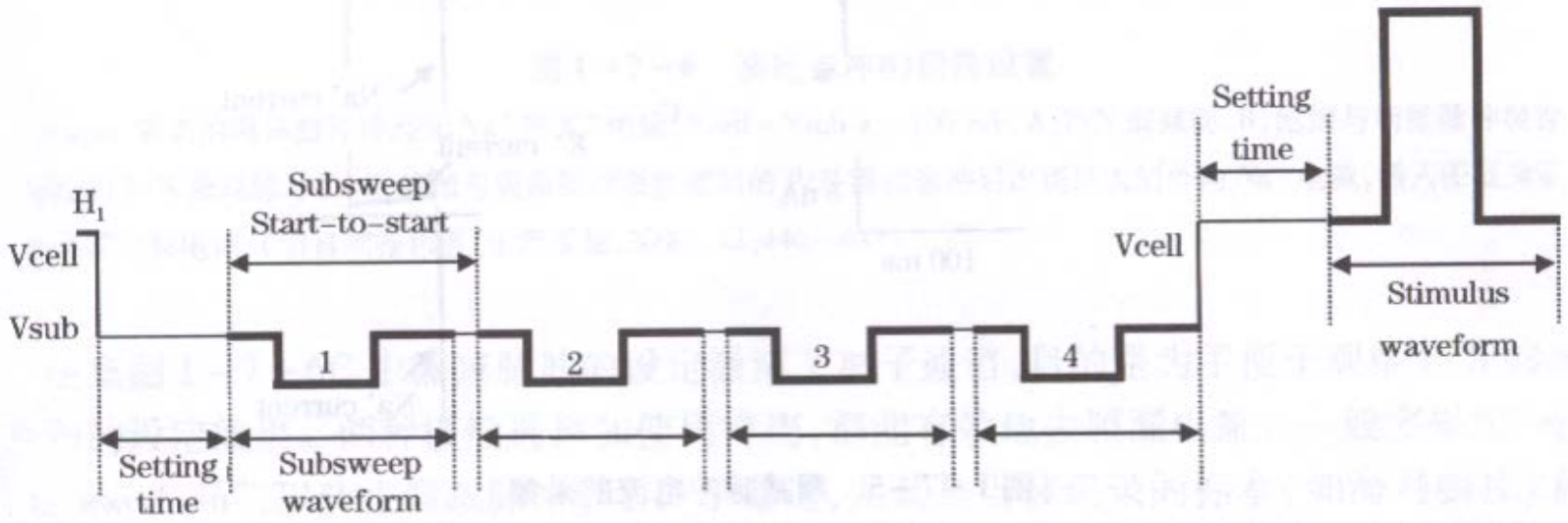
Passive current 包括transient膜电容电流和steady-state

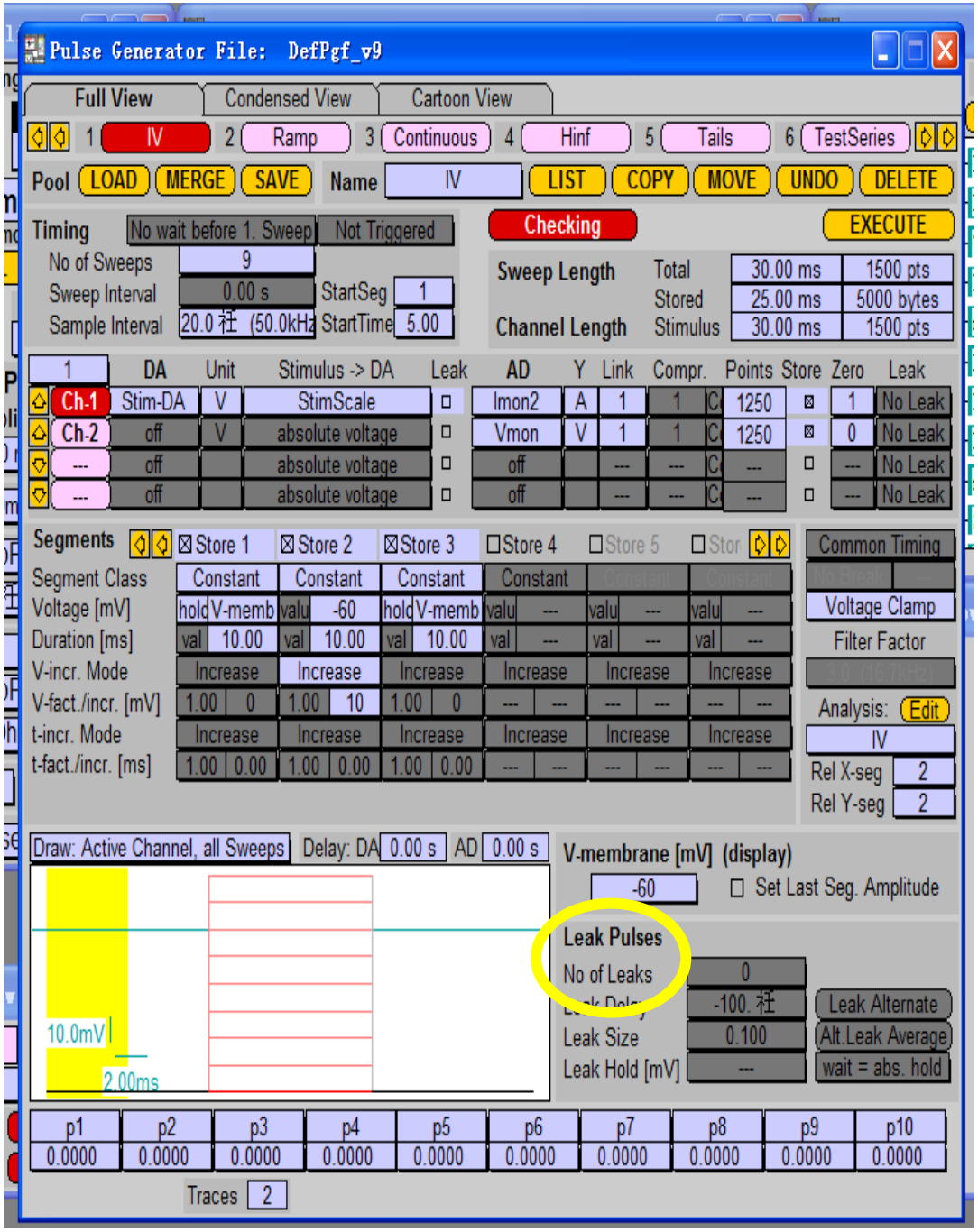
P/N（positive/negative)主要用来去掉steady-state current
（通常只用于电压门控的离子通道）



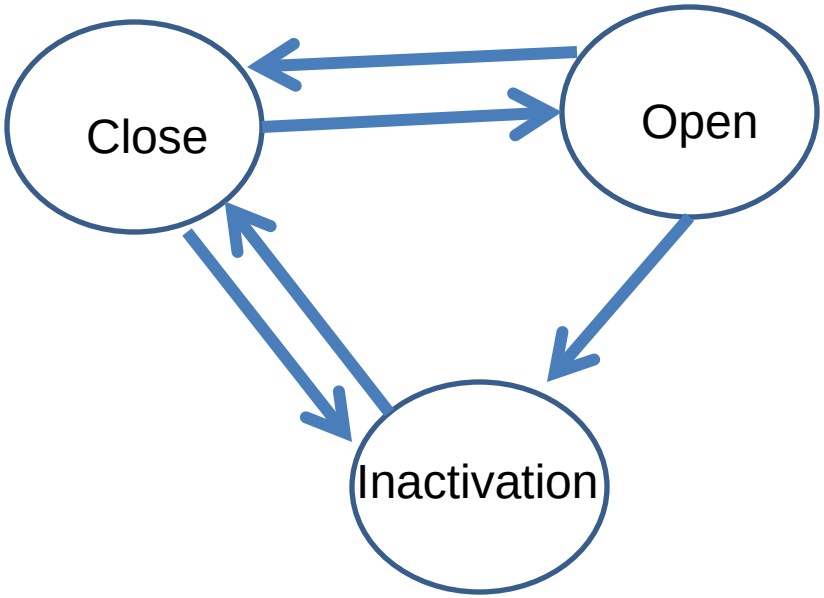
一般用P/4,反方向


Edit Protocol - NaCN-ramp.pro
Mode/Rate
Inputs
Outputs
Trigger
Statistics
Comments
Math
Waveform
Stimulus
Waveform Analog OUT: Cmd 0
Info
Pre-sweep Train
Number of pulses in train: 1
Pulse frequency = 500 Hz Train duration = 2 ms
Baseline duration (ms): 1
Step duration (ms): 1
Post-train duration (ms): 10
Baseline level (mV): 0
Step level (mV): 0
Post-train level (mV): 0
P/N Leak Subtraction
Apply to Analog IN signal: IN 0
Number of subsweeps: 4
Subsweep start-to-start (ms): Minimum
Settling time (ms): 100
Execution
Before
After
Polarity
Same as waveform
Opposite to waveform
Subsweep holding level (mV): 0
Show corrected sweep data
User List
Parameter to change: Number of pulses in pre-sweep train
List of parameter values:
Repeat
Membrane Test Between Sweeps
Settling time (ms): Before 100 After 100 For other settings, use Configure / Membrane Test Setup
Channel #0
Channel #1
Channel #2
Channel #3
OK
Cancel
Help
Acquisition mode:
Episodic stimulation
Update Preview


H1
Vcell
Vsub
Subsweep
Start-to-start
1
2
3
4
Setting
time
Subsweep
waveform
Vcell
Setting
time
Stimulus
waveform


Pulse Generator File: DefPgf_v9
Full View
Condensed View
Cartoon View
1 IV
2 Ramp
3 Continuous
4 Hinf
5 Tails
6 TestSeries
Pool LOAD MERGE SAVE
Name IV
LIST COPY MOVE UNDO DELETE
Timing No wait before 1. Sweep
Not Triggered
No of Sweeps 9
Sweep Interval 0.00 s
StartSeg 1
Sample Interval 20.0 社 (50.0kHz
StartTime 5.00
Checking
EXECUTE
Sweep Length Total 30.00 ms 1500 pts
Stored 25.00 ms 5000 bytes
Channel Length Stimulus 30.00 ms 1500 pts
1 DA Unit Stimulus -> DA Leak AD Y Link Compr. Points Store Zero Leak
Ch-1 Stim-DA V StimScale Imon2 A 1 1 C 1250 1 No Leak
Ch-2 off V absolute voltage Vmon V 1 1 C 1250 0 No Leak
--- off absolute voltage off --- --- C --- --- No Leak
--- off absolute voltage off --- --- C --- --- No Leak
Segments
Store 1
Store 2
Store 3
Store 4
Store 5
Stor
Common Timing
Segment Class Constant Constant Constant Constant Constant Constant
Voltage [mV] hold V-memb valu -60 hold V-memb valu --- valu --- valu ---
Duration [ms] val 10.00 val 10.00 val 10.00 val --- val --- val ---
V-incr. Mode Increase Increase Increase Increase Increase Increase
V-fact./incr. [mV] 1.00 0 1.00 10 1.00 0 --- --- --- --- --- ---
t-incr. Mode Increase Increase Increase Increase Increase Increase
t-fact./incr. [ms] 1.00 0.00 1.00 0.00 1.00 0.00 --- --- --- --- --- ---
Voltage Clamp
Filter Factor
Analysis: Edit
IV
Rel X-seg 2
Rel Y-seg 2
Draw: Active Channel, all Sweeps
Delay: DA 0.00 s AD 0.00 s
10.0mV
2.00ms
V-membrane [mV] (display)
-60
Set Last Seg. Amplitude
Leak Pulses
No of Leaks 0
-100. 社
Leak Size 0.100
Leak Hold [mV] ---
Leak Alternate
Alt.Leak Average
wait = abs. hold
p1 p2 p3 p4 p5 p6 p7 p8 p9 p10
0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000
Traces 2

电压门控离子通道的特性



Activation depolarization -80------20

Inactivation

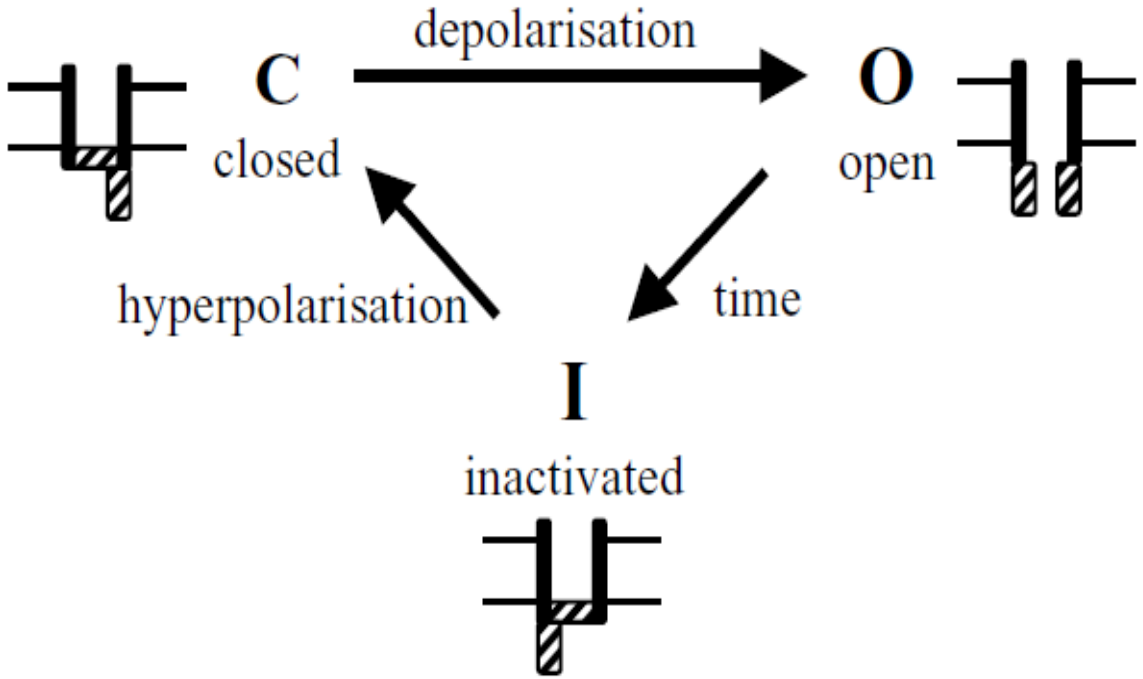
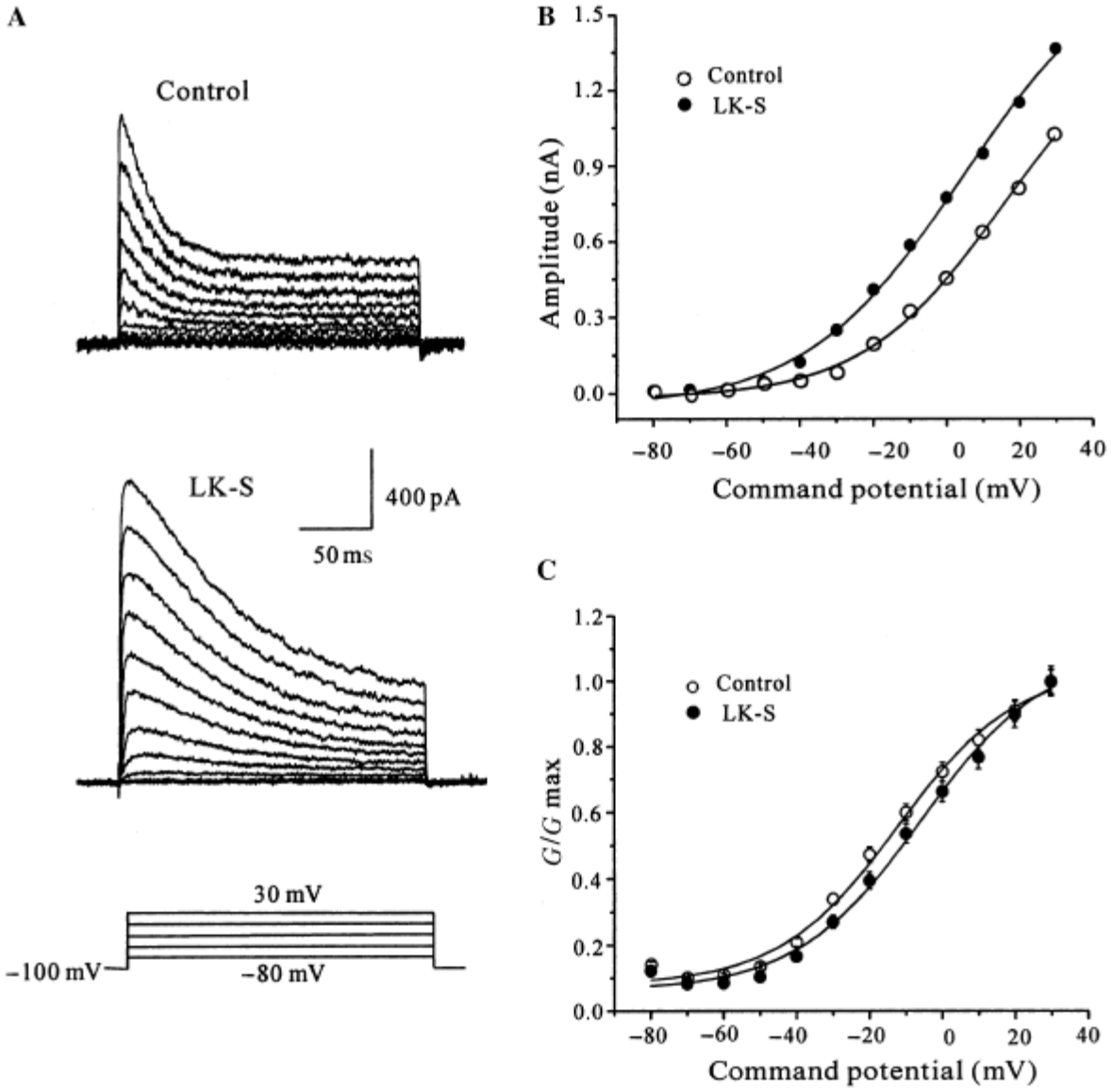


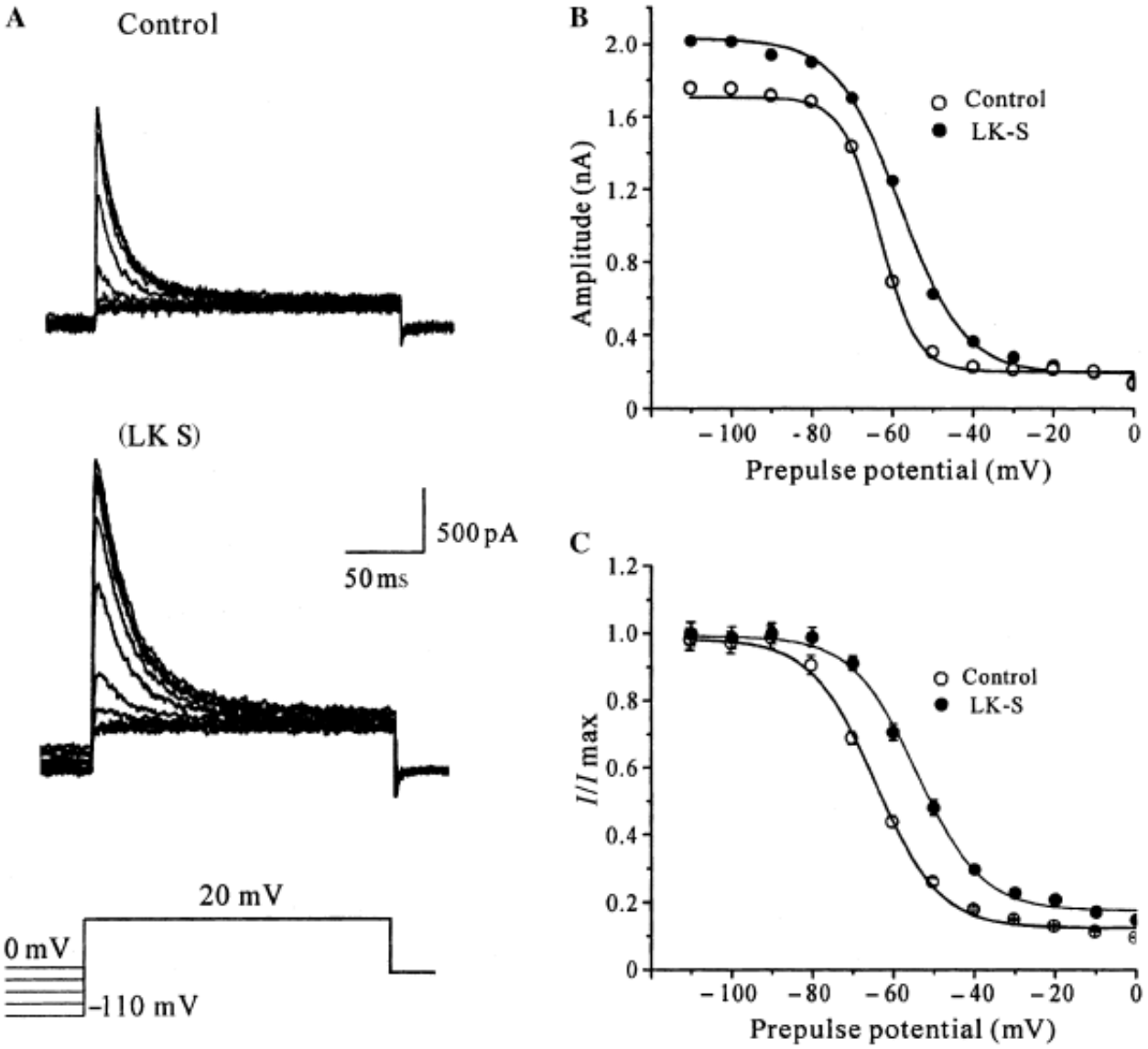
Figure 5.18 A sodium channel has separate activation and inactivation gates operated by membrane voltage and time

问题5：稳态激活与失活曲线



Activation Curve

inactivation Curve



窗电流

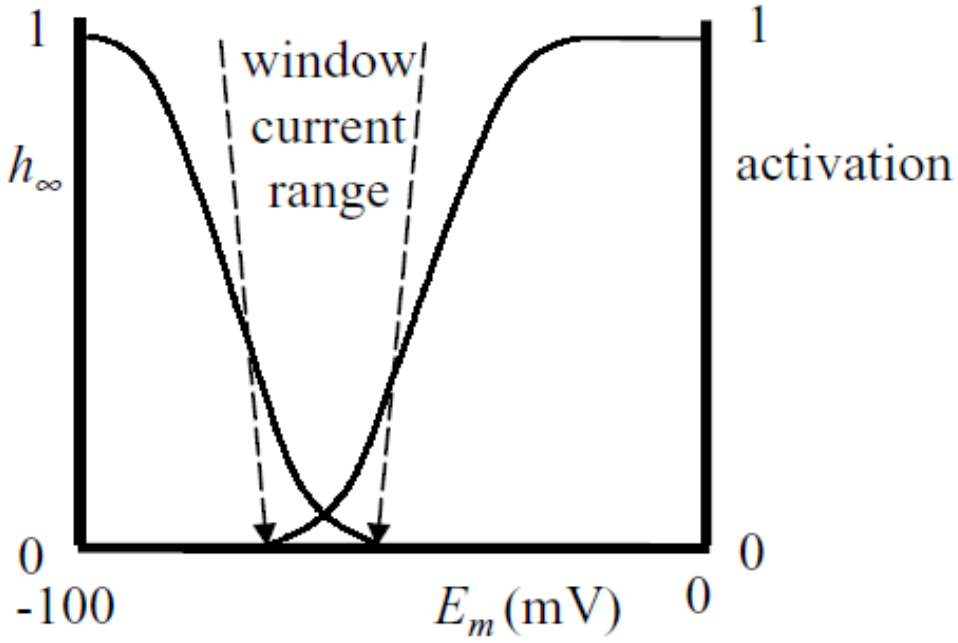
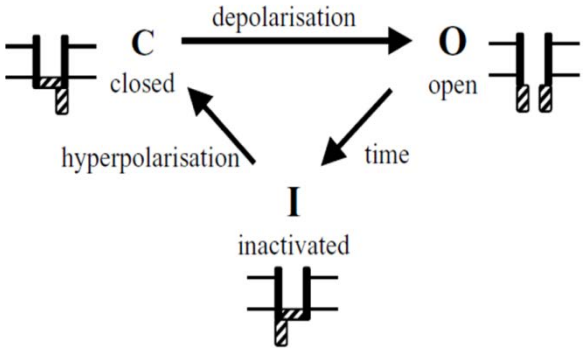
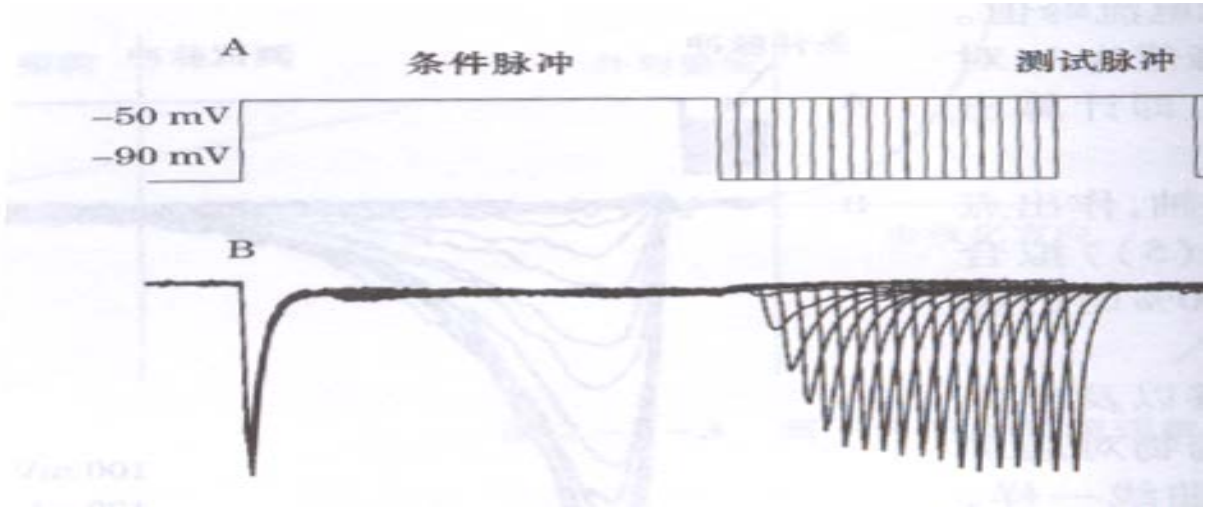


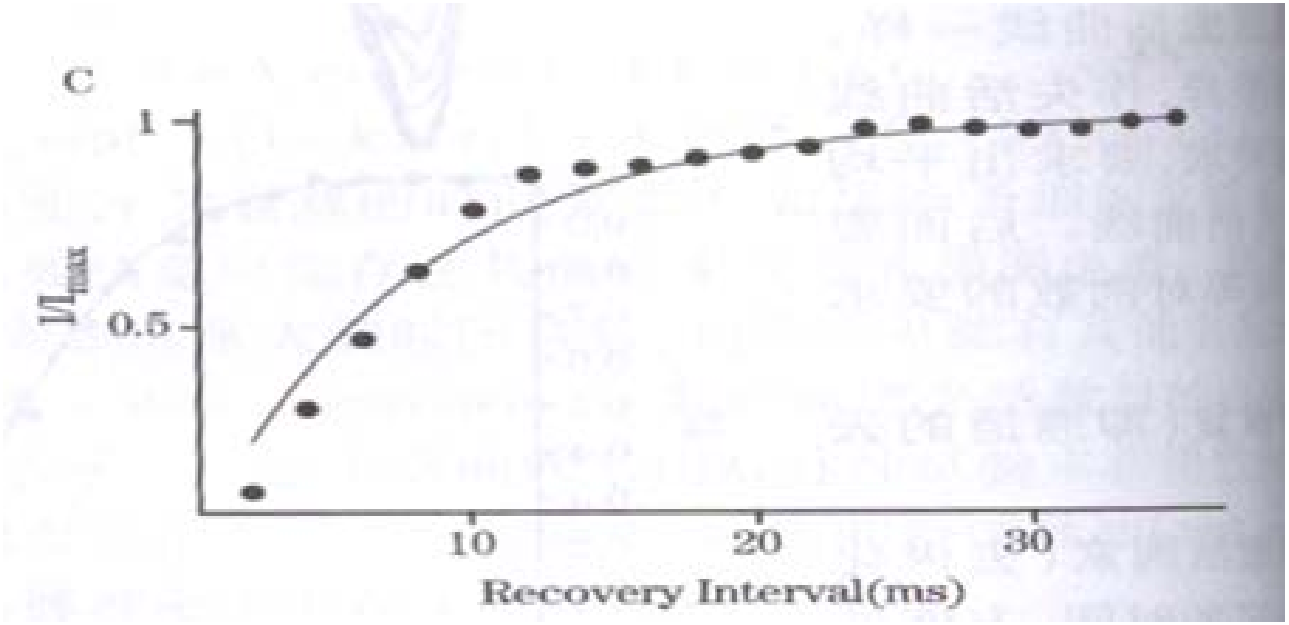
Figure 5.20 Overlap between activation and inactivation curves creates a range of membrane voltage where a small, continuous current is present

激活、失活、窗电流有什么意义？

从失活中恢复


C
closed
depolarisation
O
open
hyperpolarisation
time
I
inactivated


A
条件脉冲
测试脉冲
–50 mV
–90 mV
B


C
1
0.5
I/I_{max}
10
20
30
Recovery Interval(ms)

问题6：什么是内向外向整流（inward/outward rectifying)

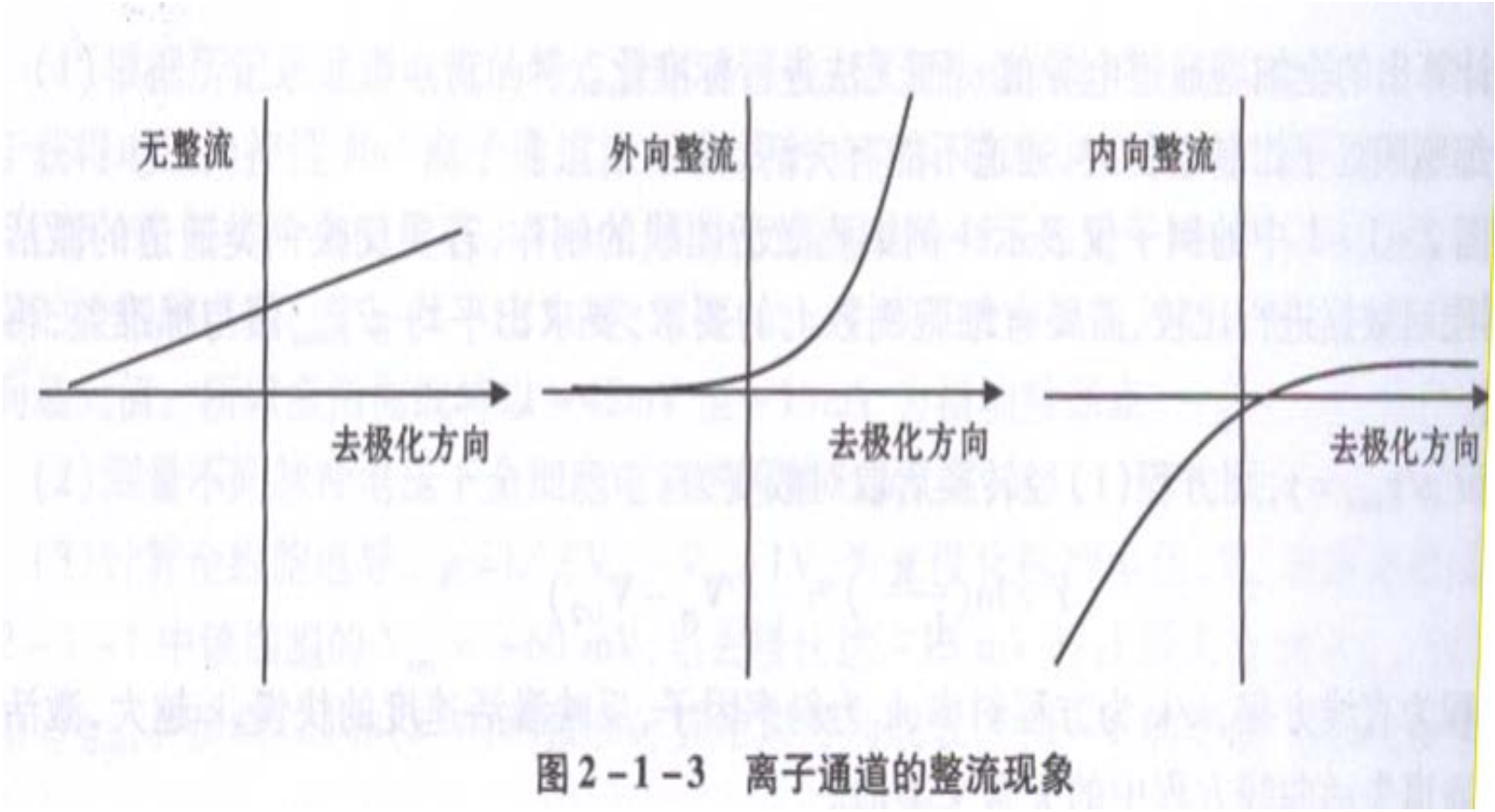


图2-1-3　离子通道的整流现象

问:7：细胞、切片的准备

1、我真的会培养细胞吗？

标准：知道什么样的细胞是健康的，知道如何让细胞配合你做Patch

细胞代数

2、脑切片的准备

解剖液与孵育液

rat CSF:
Na+ 156 mM, Cl− 126 mM, K+ 2.8 mM, **glucose** ~3.6 mM, lactate 2.8 mM, Ca2+ 2.2 mM，pH 7.35, 302 mOsmol

孵育液（解剖液、记录外液）(mM): 124 NaCl, 2.8 KCl, 2 CaCl2, 2 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, 10 D-**glucose**,
pH 7.4, 295 mOsm.

解剖液: 206 **sucrose**, 2.8 KCl, 1 CaCl2,
1 MgCl2, 2 MgSO4, 1.25 NaH2PO4, 26 NaHCO3, 10 D-glucose, pH 7.4, 295 mOsmol. （年长动物）.

不同实验室可能有其他添加：kynurenic acid（4-羟基喹啉二甲酸）
ascorbic acid

解剖液与孵育液的温度

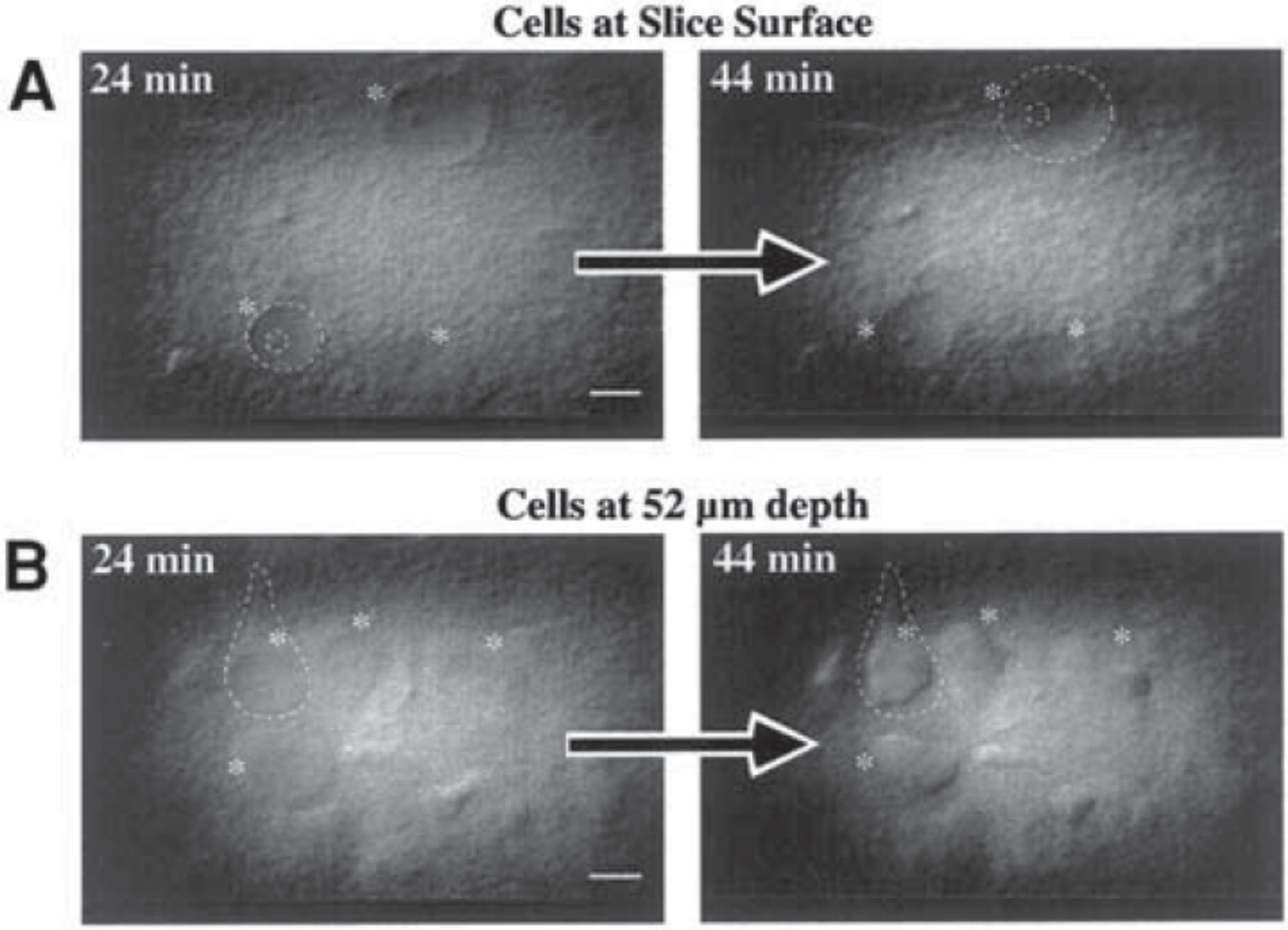
~1°C

22°C

在孵育液里恢复45分钟后再记录

35°C 预孵育需不需要？

对于相对年老的动物>4W



James R. Moyer, Jr. and Thomas H. Brown

Neuromethods, Vol. 35

问题8：干扰

I_{rms}

RMS: root mean square of the current noise

干扰应该多大？

Axon 200B





I_{rms}

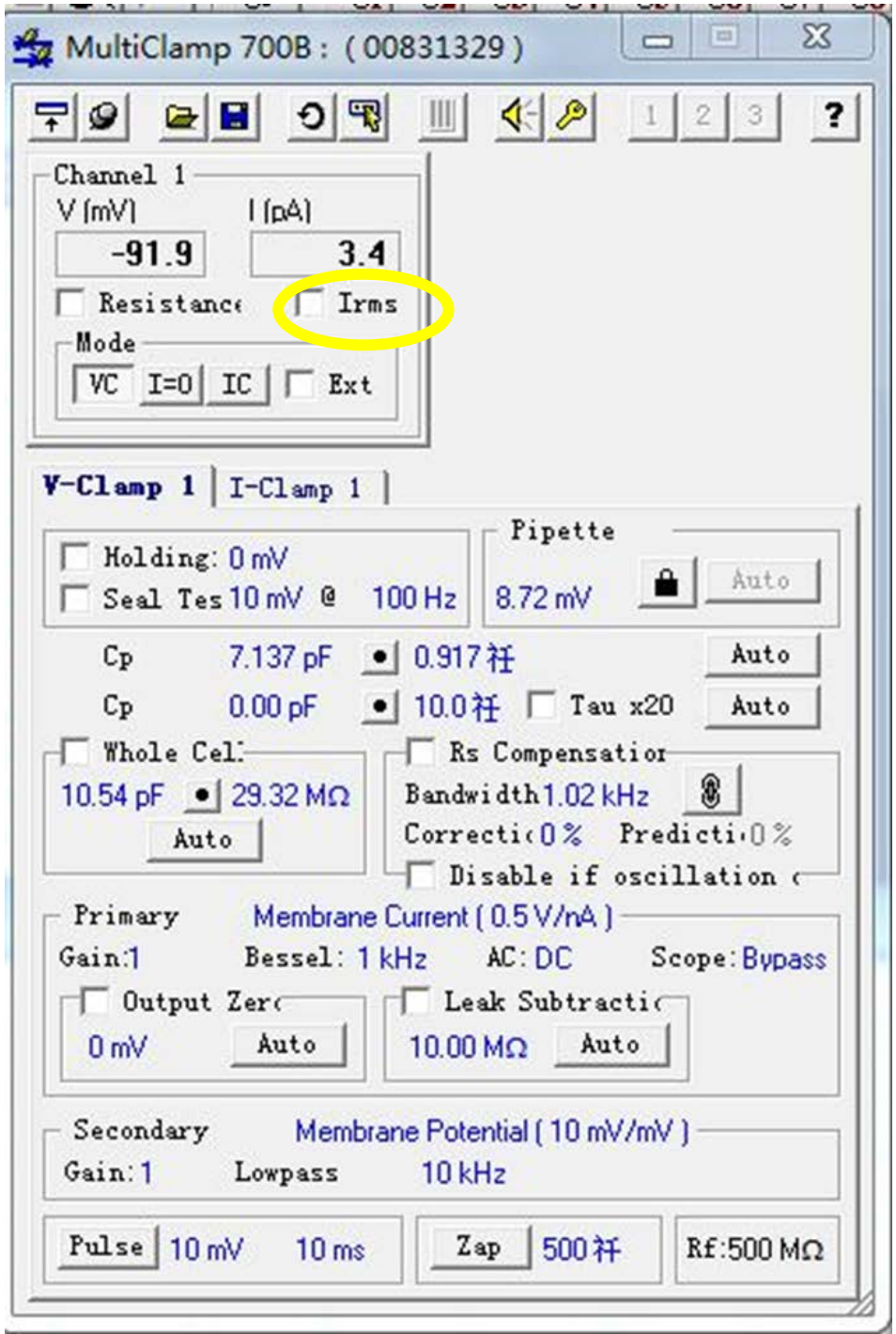
patch



全细胞 Whole cell β =1 ≈ 0.55 pA

单通道 Patch cell β =1 ≈ 0.045pA

Axon 700B



4. In the MultiClamp 700B Commander, check the "Irms" box beneath the corresponding Channel meter for the CV-7 headstage (test one headstag a time). Compare the value indicated by the meter to that listed in the ta below (*5 kHz, 4-pole Butterworth measurement).

Feedback Resistor	Noise*
50 MΩ	2.0 pA rms
500 MΩ	0.8 pA rms
5 GΩ	0.5 pA rms
50 GΩ	0.15 pA rms

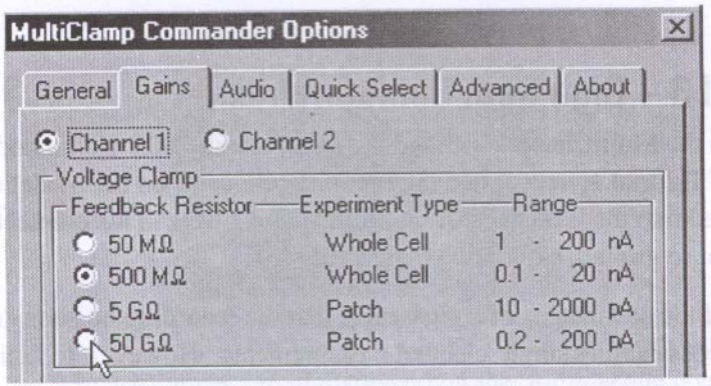
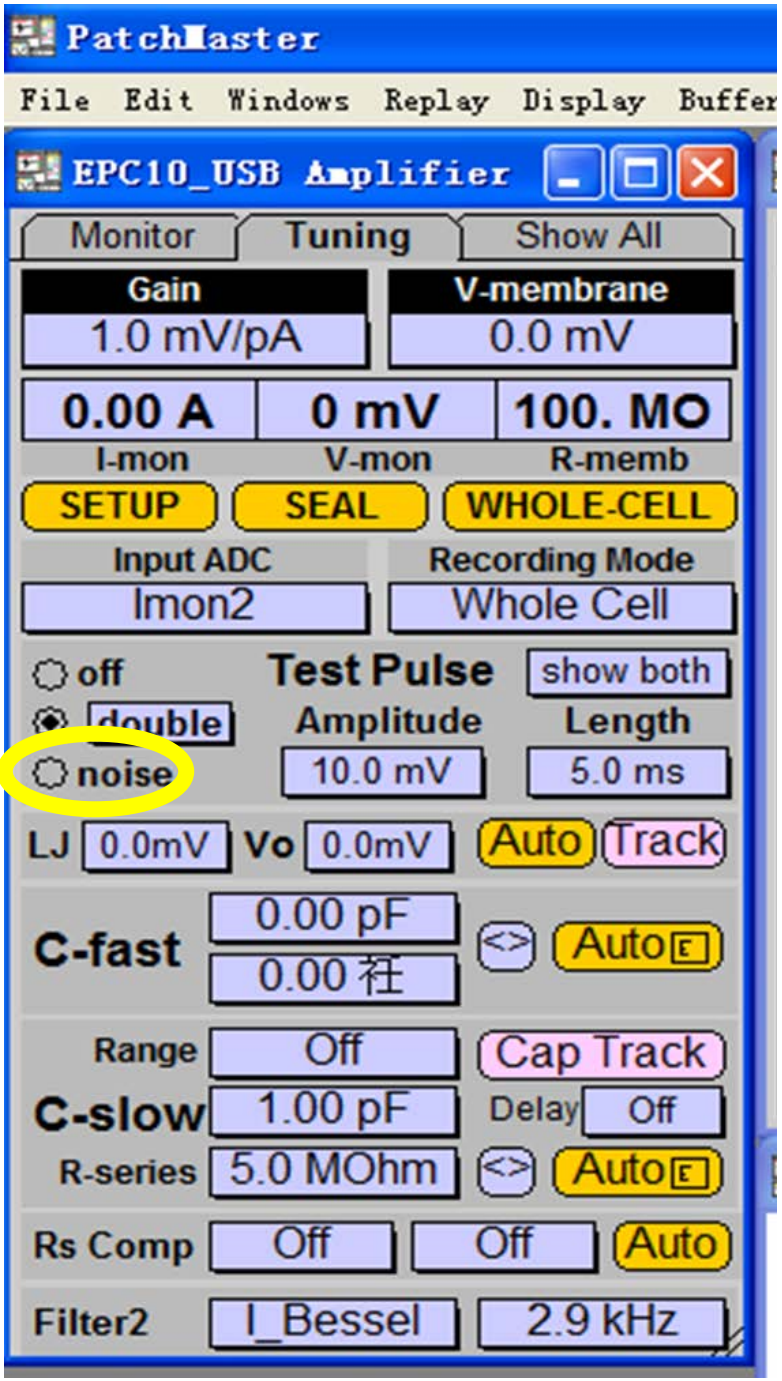


Figure 3.10

50G resistor : 0.11pA



干扰的来源：一切可能的电磁场和震动

震动：防震台(轻轻跺脚）

排除干扰的方法：接地

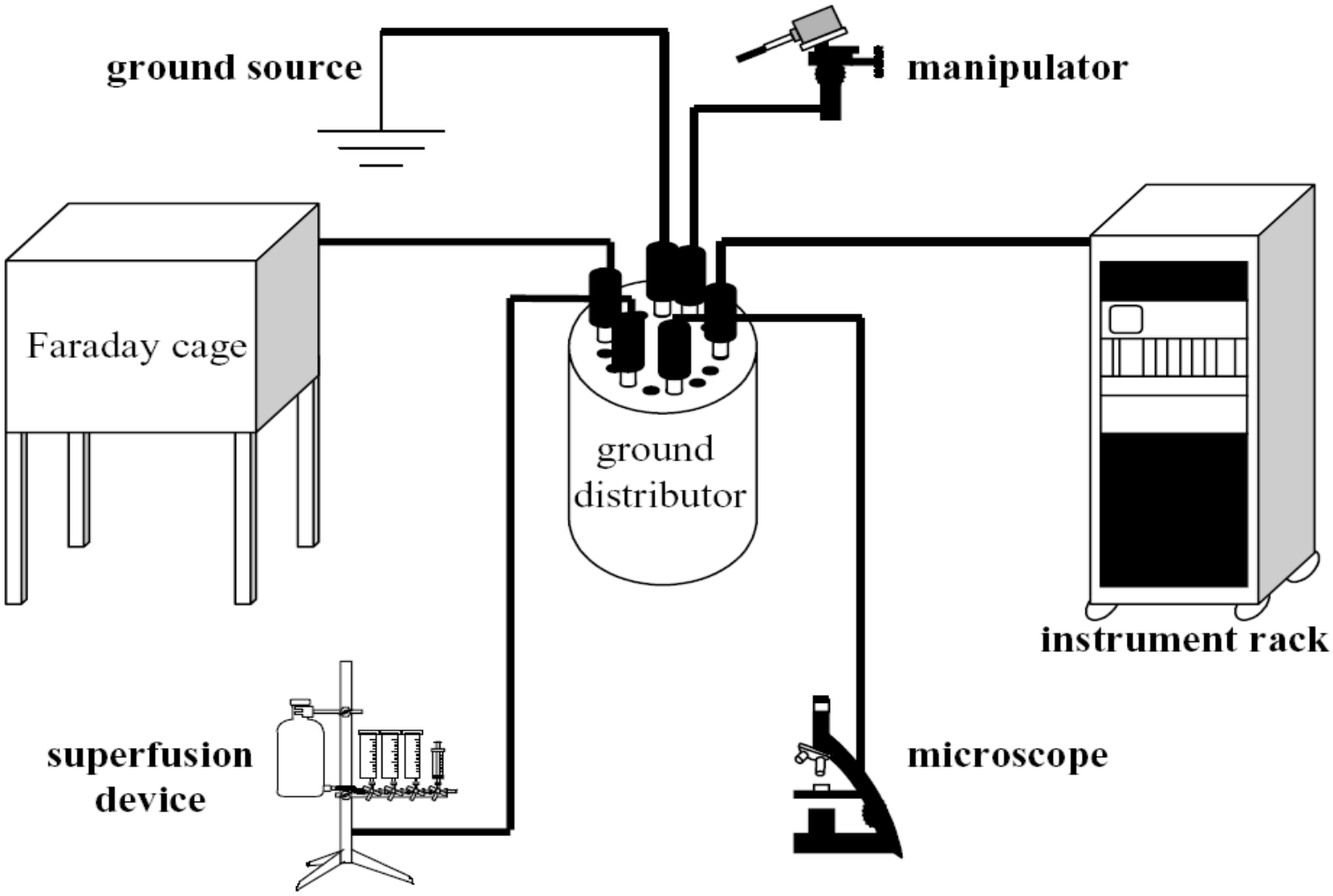


Figure 3.21 A ground distributor in the shape of a metal block or strip prevents ground loops

外部噪音

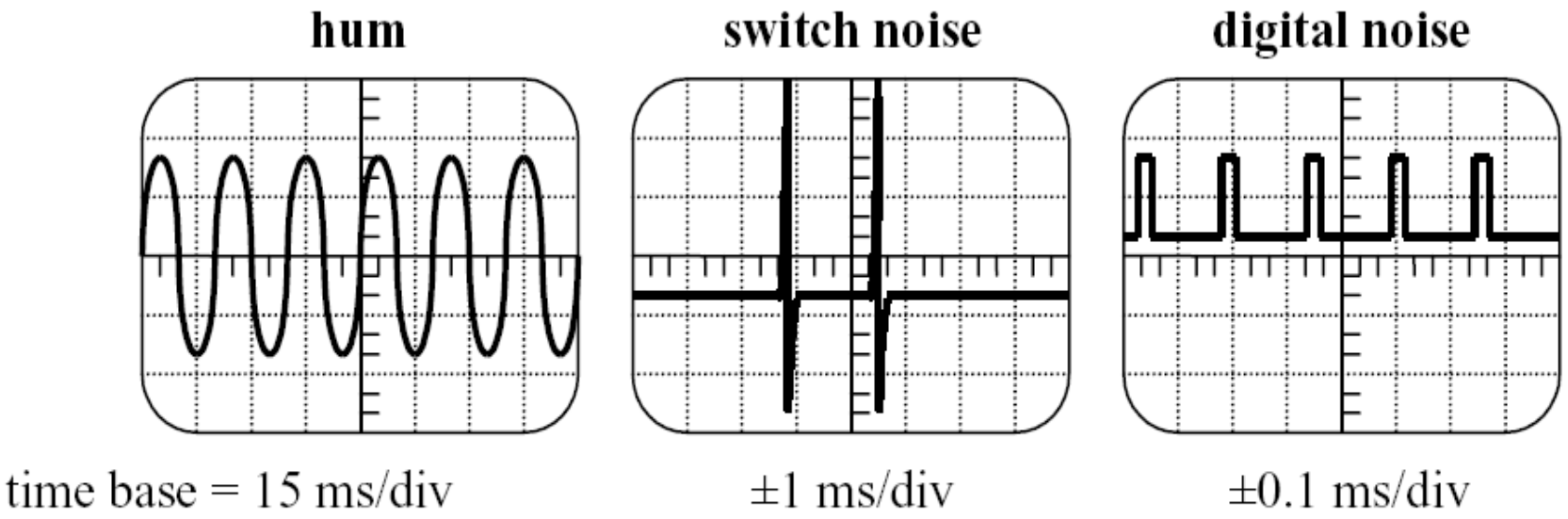
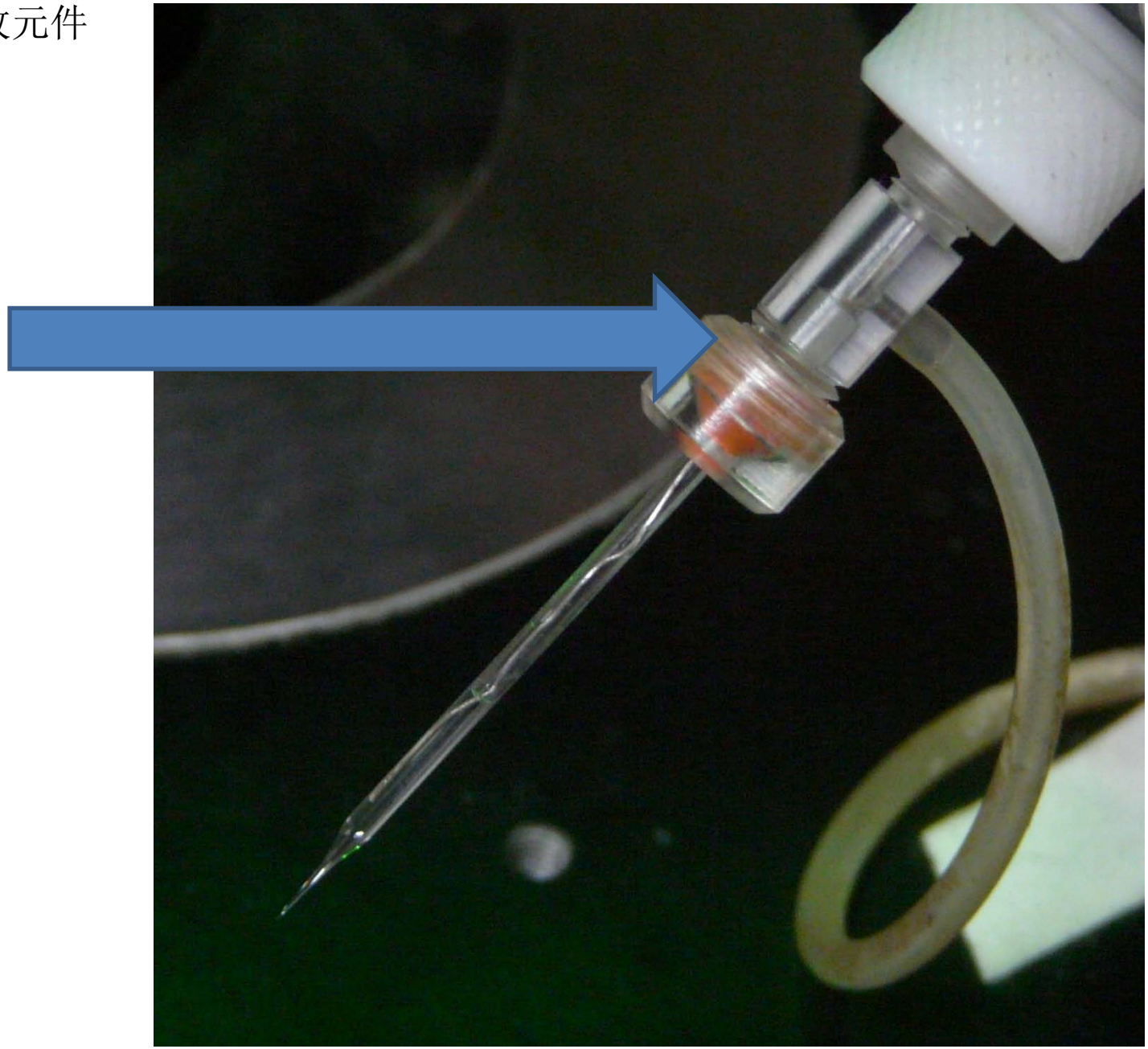


Figure 3.20 The appearance on an oscilloscope of common types of external noise

Hum is caused by the power grid alternating current, with a frequency of 50 Hz in Europe and 60 Hz in North America.

噪音的最大接收元件

保持holder干净



内在噪音

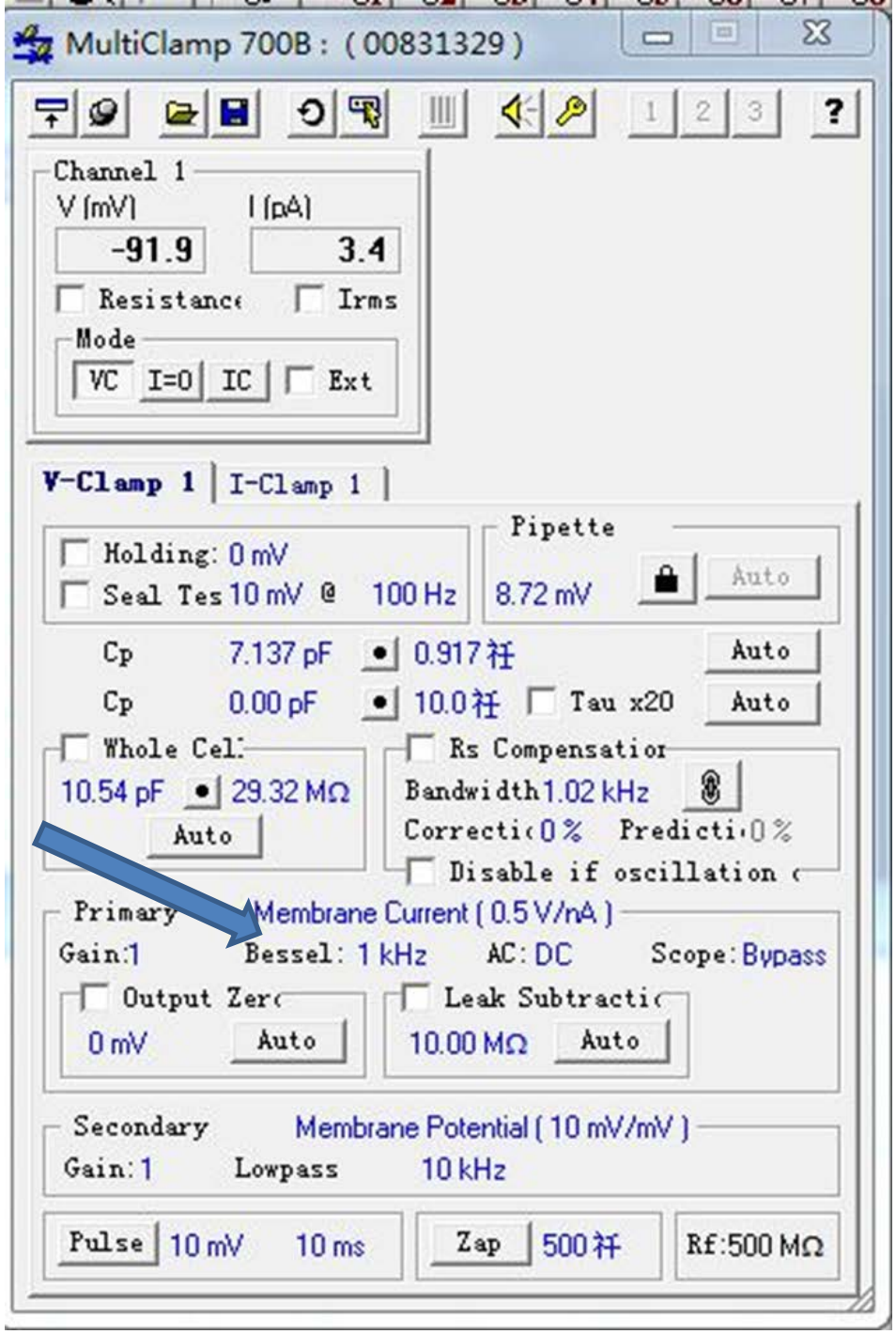
热量、电极电容、不需要的其他信号。。。。

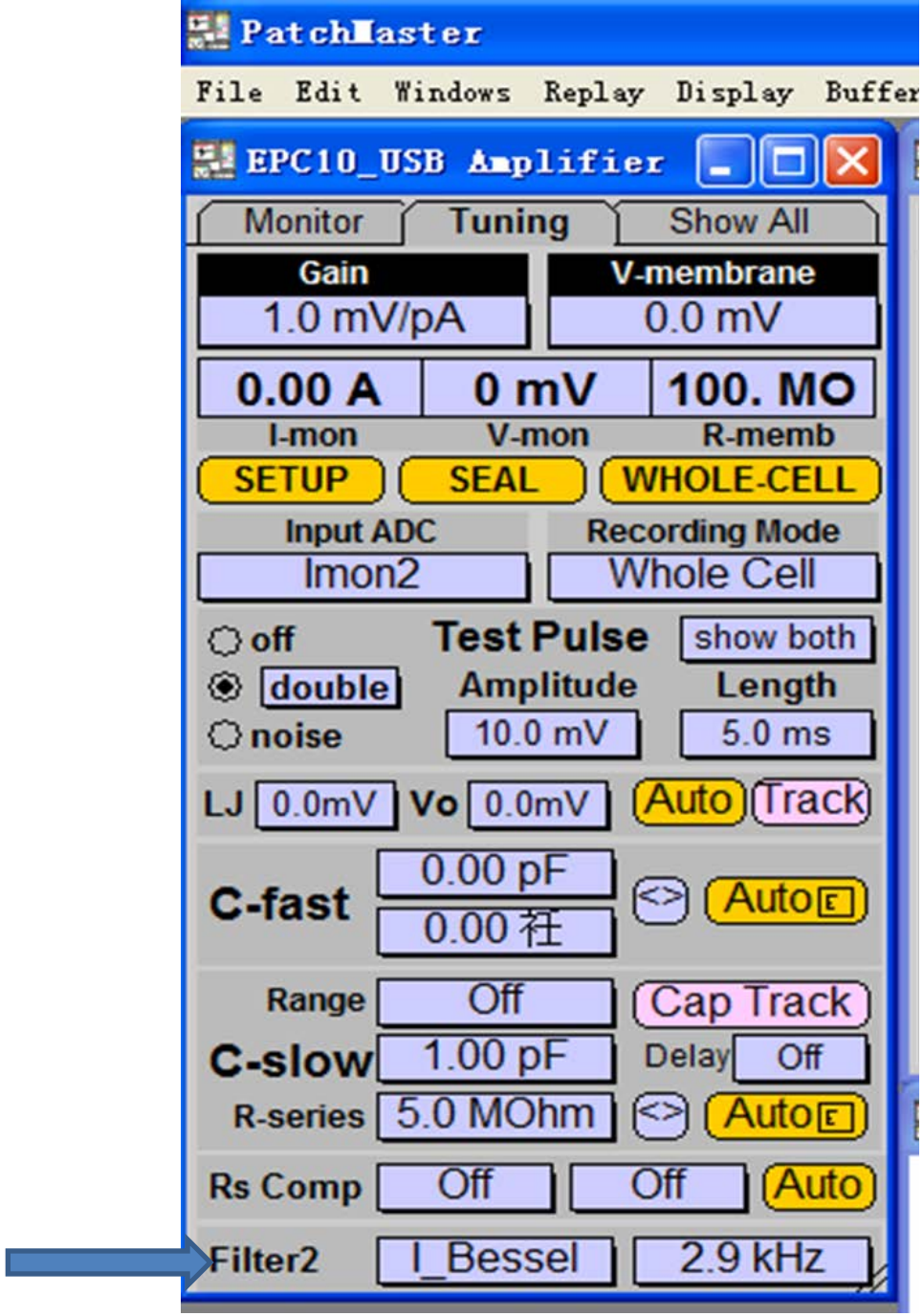
Filter：refer to frequency

Low pass filter: 1K\2K\5K

Low pass bessel filter




MultiClamp 700B : (00831329)
Channel 1
V (mV)
I (pA)
-91.9
3.4
Resistance
Irms
Mode
VC
I=0
IC
Ext
V-Clamp 1
I-Clamp 1
Holding: 0 mV
Seal Tes
10 mV @ 100 Hz
Pipette
8.72 mV
Auto
Cp 7.137 pF 0.917 µs Auto
Cp 0.00 pF 10.0 µs Tau x20 Auto
Whole Cel
10.54 pF 29.32 MΩ
Auto
Rs Compensation
Bandwidth 1.02 kHz
Correcti 0 %
Predicti 0 %
Disable if oscillation
Primary
Membrane Current (0.5 V/nA)
Gain:1
Bessel: 1 kHz
AC: DC
Scope: Bypass
Output Zero
0 mV
Auto
Leak Subtracti
10.00 MΩ
Auto
Secondary
Membrane Potential (10 mV/mV)
Gain: 1
Lowpass
10 kHz
Pulse
10 mV
10 ms
Zap
500 µs
Rf:500 MΩ


PatchMaster
File Edit Windows Replay Display Buffer
EPC10_USB Amplifier
Monitor
Tuning
Show All
Gain
1.0 mV/pA
V-membrane
0.0 mV
0.00 A
0 mV
100. MO
I-mon
V-mon
R-memb
SETUP
SEAL
WHOLE-CELL
Input ADC
Imon2
Recording Mode
Whole Cell
off
double
noise
Test Pulse
Amplitude
10.0 mV
Length
5.0 ms
show both
LJ
0.0mV
Vo
0.0mV
Auto
Track
C-fast
0.00 pF
0.00 pF
Auto
Range
Off
Cap Track
C-slow
1.00 pF
Delay
Off
R-series
5.0 MOhm
Auto
Rs Comp
Off
Off
Auto
Filter2
I_Bessel
2.9 kHz

Data acquisition and digitisation (AD)

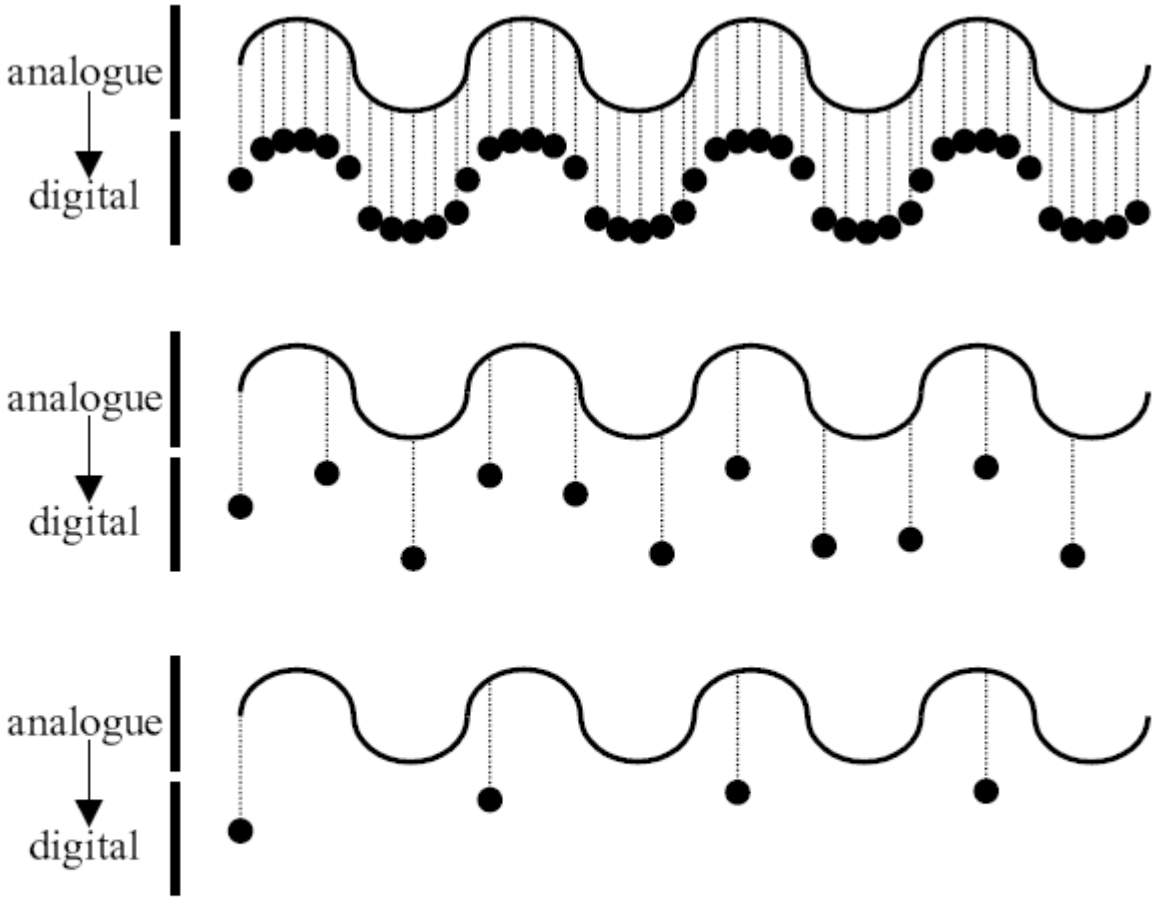


Figure 3.28 Effect of different sampling rates on quality of rendition of an analogue signal. The top panel shows sampling that results in a reasonable representation of the analogue signal. The low sampling rate in the middle panel results in a severely distorted digital signal. The bottom panel demonstrates aliasing: the appearance of artefacts in the form of new (slower) waveforms when the sampling rate is grossly inadequate

Sampling rate:
At least 2X filter setting

例如：filter is 2K， Sampling should be 5K or more

