Menicon

Instruction Manual

For ShellPa Pro (Model type : SSMP)

Lifescience Dept. Menicon Co.,Ltd.

＜Table of contents＞

1. Preparing the equipment

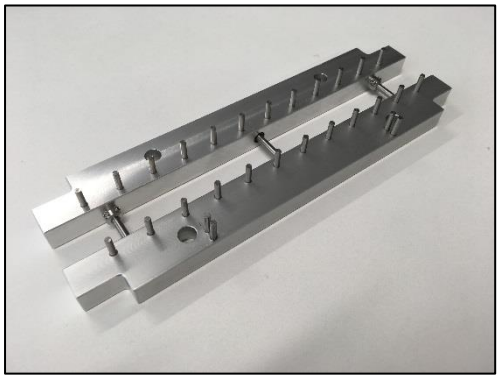
1.1. Checking the supplied items

First, please check, there are below units and parts.

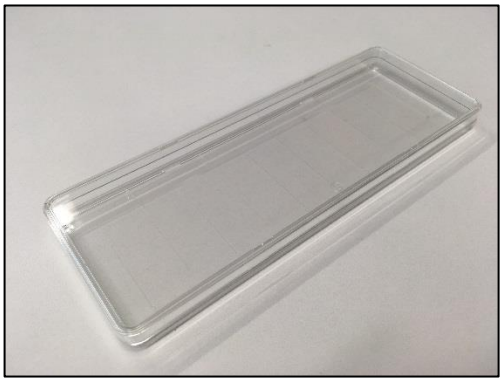
Main unit ×1unit

Control unit ×1 unit

Stretch chamber holder ×1holder

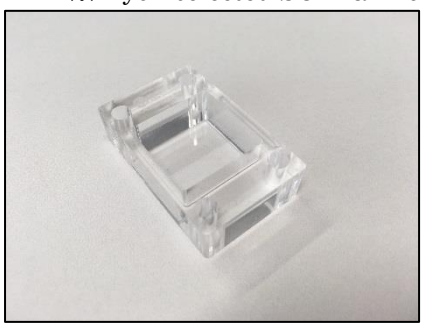


Cover plate ×5plates



Stretch chamber SC4Dea ×1pack(10 chamber)

※If you selected SC4Ha in order, we could supply SC4Ha×1pack(10 chamber).

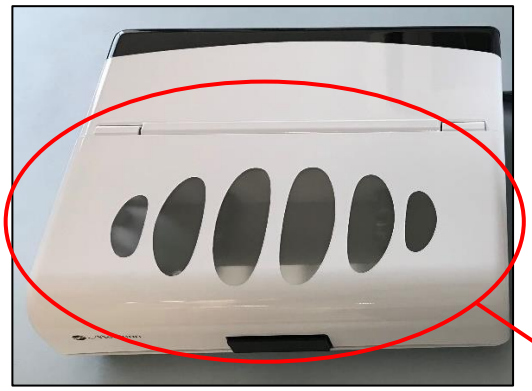


Power supply adaptor ×1

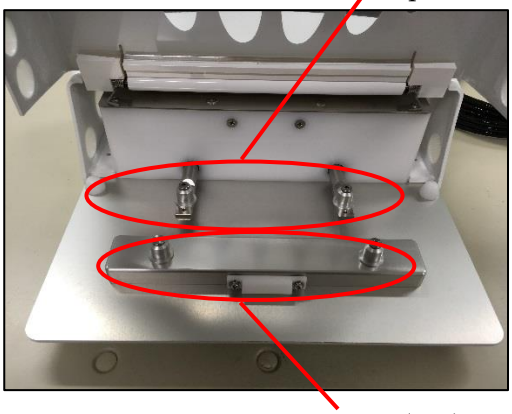
Power supply cable ×1

1.2. Identifying parts

<Main unit>



Access cover



Stretch and relax point (bar)

Rigid point (bar)

Connecting cable 凸

<Control unit>

Main switch

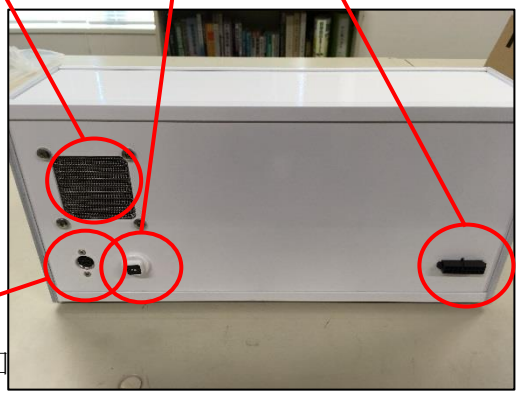
Main display

Power
cable connector 凹

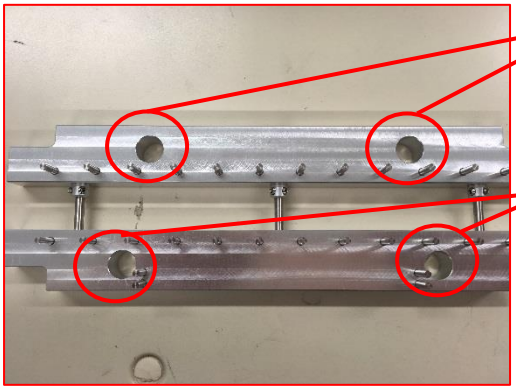
Vent hole

Fuse

Connecting cable 凹



<Stretch chamber holder>



Setting hole for movement point (bar)
(Distance between both bar, is close.)

Setting hole for rigid point (bar)
(Distance between both bar, is far.)

1.3. Setting and connecting of each item

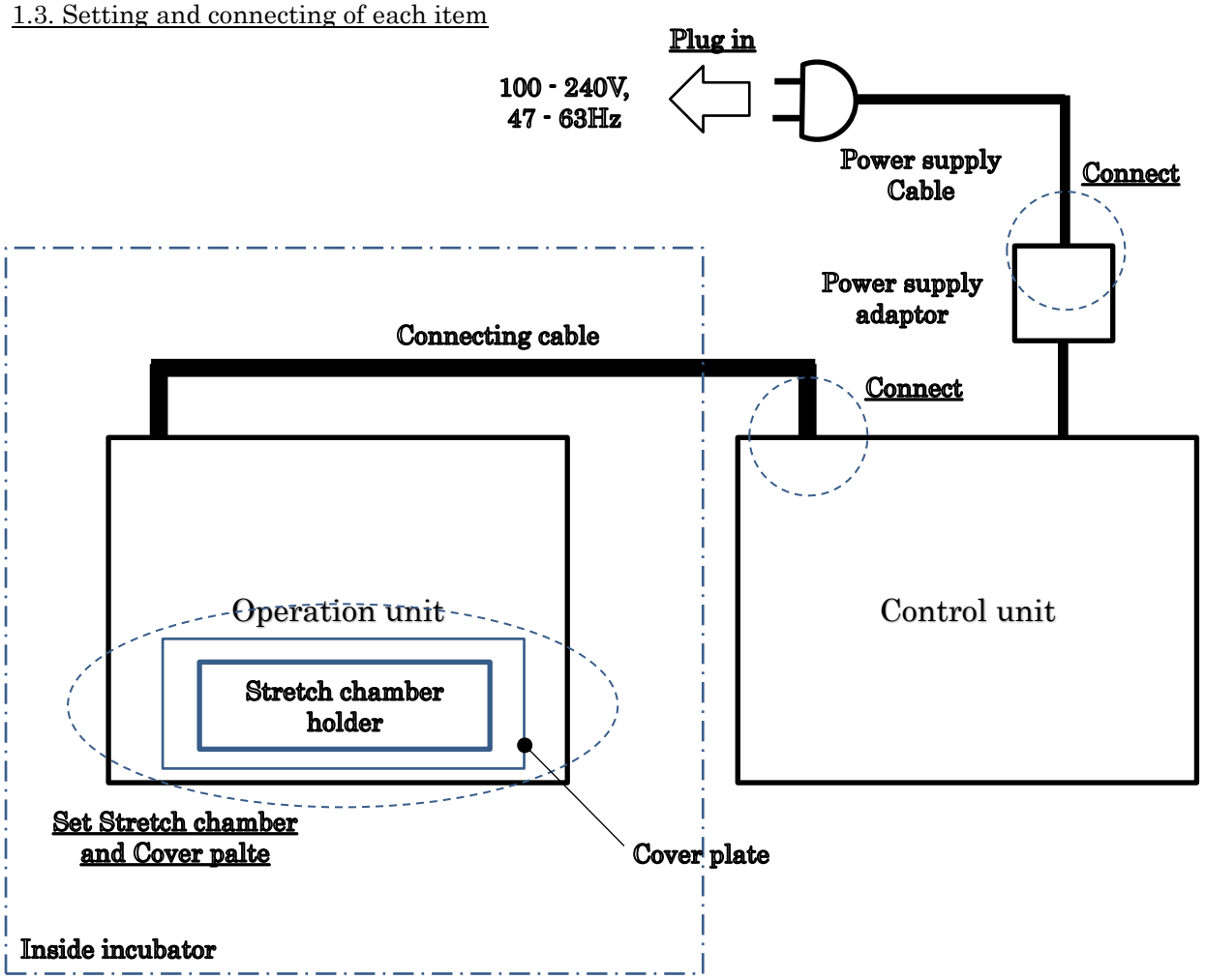


Figure1.Connecting and setting ShellPa Pro

Beforehand, cleaning up closely, inside and outside of “operation unit” and “stretch chamber holder”, using ethanol. After, install it into incubator.

Like figure1, please connect each equipment, and plug in power supply. (But this time, not set “Stretch chamber holder” and “cover plate” on “operation unit”.)

1.4. Switch on and check initial position (original point).

Below procedure, please check and conduct.

Beforehand, please check nothing of an obstacle in chamber holder setting area on “Operation unit”.--- [1]

↓

Please turn on a “main switch” on “Control unit”, if nothing. --- [2]

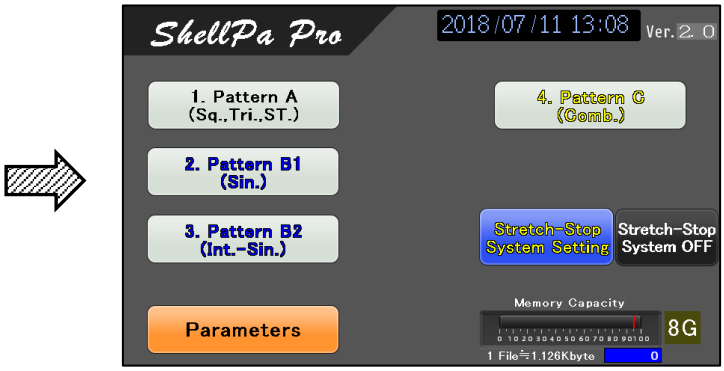
↓ (Initial set up movement on “Operation unit” start. (start to move in order to return to origin.))

After finishing to return to origin, normally, “Operation unit” will stop, and “Main menu” screen on “Main menu” of “Control unit”, will be displayed, automatically. --- [3]

[1] Check nothing of obstacles

[2] Switch on

Demo screen (automatically change)



[3] “Main menu” screen

1.5. Interface layout and Summary of each interface

<Interface layout>

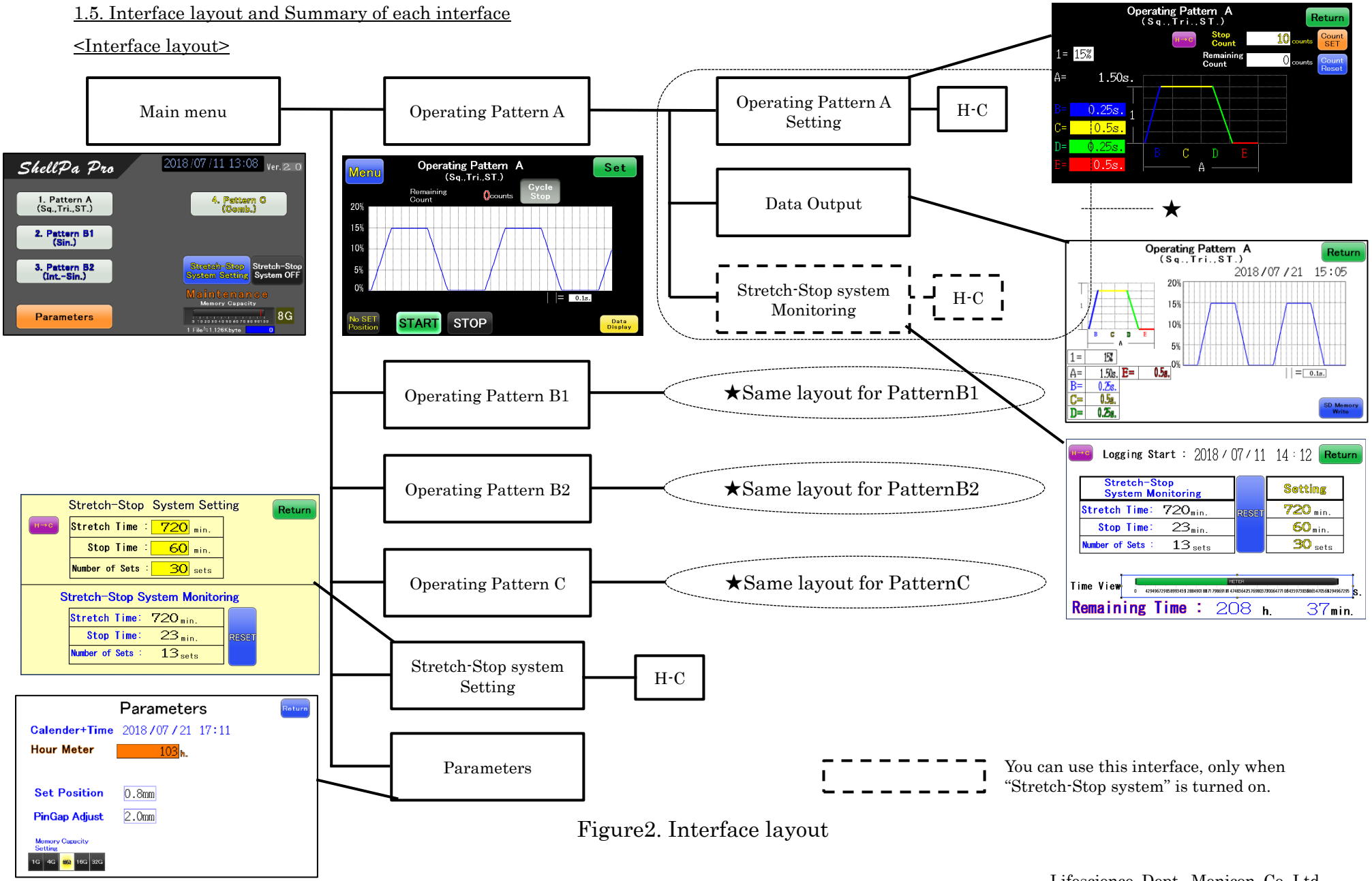


Figure2. Interface layout

<Summary of each interface>

(Main menu):

Select of stretch and relax pattern, and switching “Stretch-Stop system” mode on and off.

Other, enter to “Stretch-Stop system setting” interface or “parameter setting” interface.

(Operating Pattern A):

Start and Stop operation of stretch and relax movement in Pattern A.

(Operating Pattern A Setting):

Setting parameter of stretch and relax movement in Pattern A.

(Data Output):

Output data (parameter, wave record etc.) of stretch and relax movement in Pattern A.

(Stretch-Stop system Monitoring):

Watching each time(“Stretch time” , “Stop time”, “Number of sets”) on “Stretch-Stop system”.

And reset all counts of “Stretch-Stop system”.

(H-C): Calculation to covert time into cycle counts.

(Stretch-Stop system Setting):

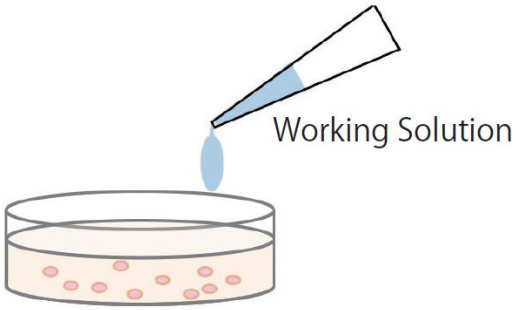
Setting and Checking each time(“Stretch time” , “Stop time” , “Number of sets”) on “Stretch-Stop system”. And reset all counts of “Stretch-Stop system”.

(Parameters):

Setting Calender + Time, and “Set Position” and “Pin Gap adjust” etc.

2. Preparing for stretch culture (Seeding on stretch chamber)

Before stretch cell culture start, you need to prepare for cell seeding on and cell attaching to “Stretch chamber”.



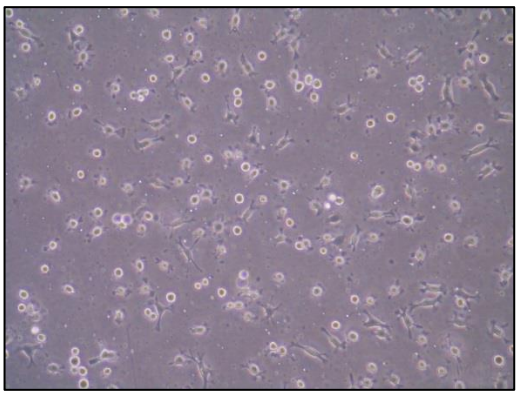
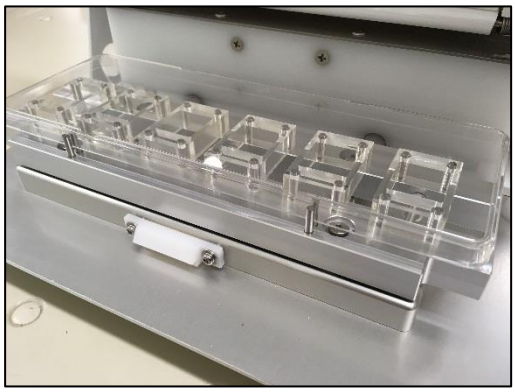


Photo: Cell seeding on and cell attaching to “Stretch chamber” in Menicon Co., Ltd. (Cell: L929, Chamber: SC4Dea, Check time: 24h. after seeding).

You can use “SC4Dea” or “SC4Ha”. We recommend quantity of culture medium is from 1.5mL to 2.0mL (In the case of induction of differentiation or long-term culture, recommended quantity’s 2.5mL.).

Set “stretch chamber” (already conducted seeding and culture in) on “stretch chamber holder”.
Next, set “Stretch chamber holder” and “Cover plate” on “Main unit”.



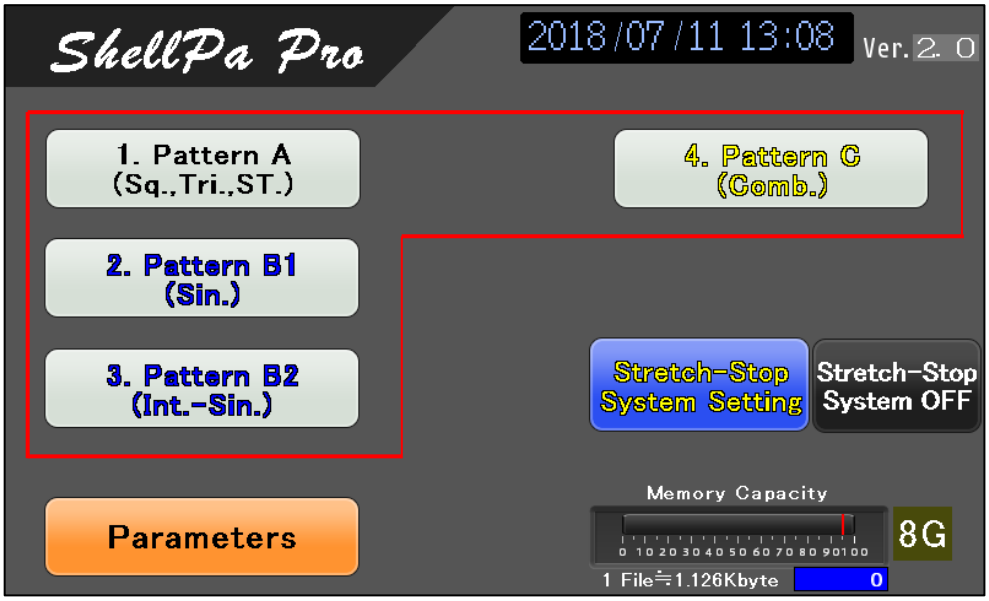
3. Basic function and operation

3.1. Selecting stretch pattern

On “ShellPa Pro”, you can practice operation of some stretch and relax patterns.

Select of each stretch and relax pattern is done in “Main menu” screen.

(Please check area inside the red line in “Main menu” screen below.)



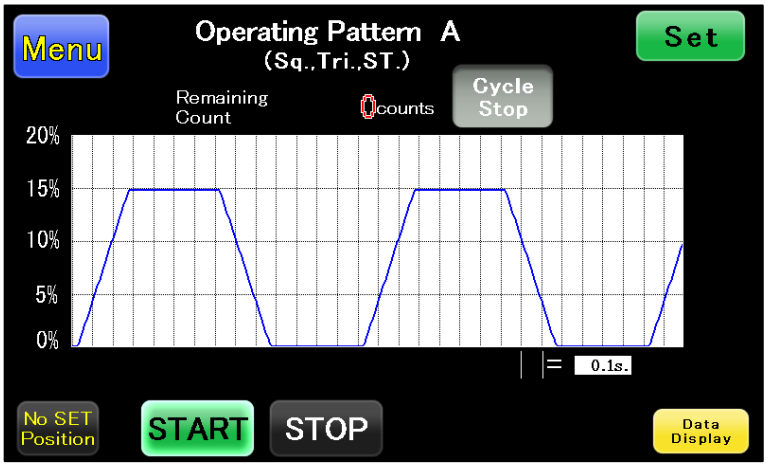
Please check below explanation of each pattern.

<Pattern A (Sq., Tri., ST.): Square wave + Standstill>

You can practice stretch and relax cell culture on constant speed. And you can set standstill time.

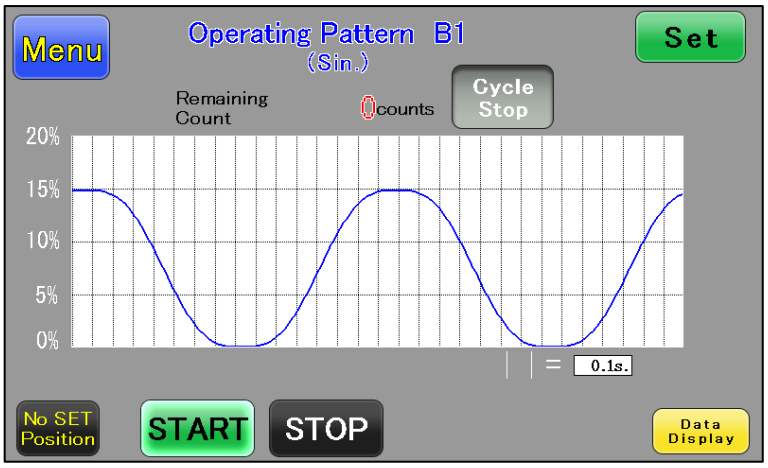
Please refer to wave on “Operating Pattern” screen below.

This pattern generally is conducted on observation of standard stretch and relax cell culture.



<Pattern B1 (Sin.): Sine wave>

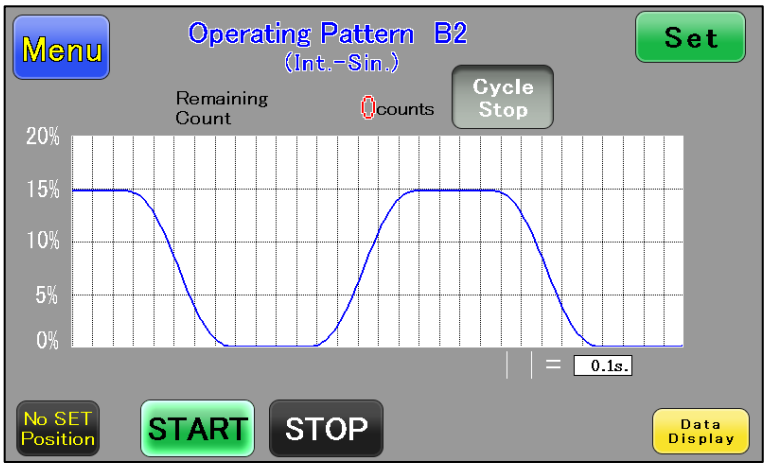
You can practice stretch frequency (sine wave frequency). This pattern is reproduction of physical beating (ex: heart beating) and movement(ex: muscle stretching and relaxing). Please refer to wave on “Operating Pattern” screen below.



<Pattern B2 (Int.-Sin.): Sine wave + Standstill>

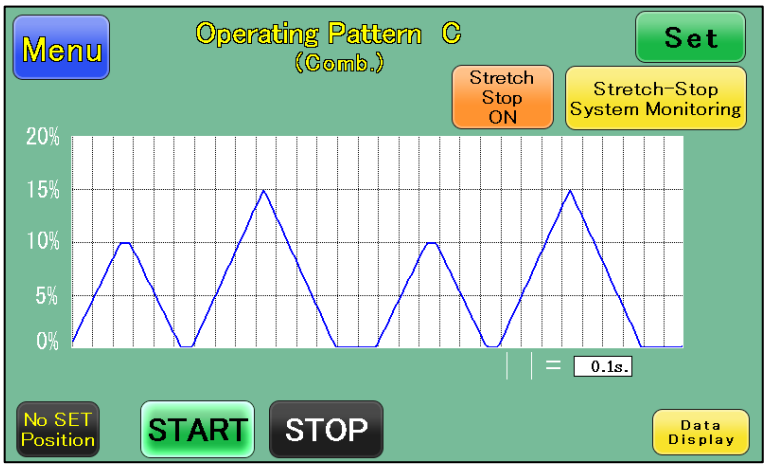
On this pattern, you can set standstill time in addition to “Pattern B1”.

If retention time set on zero, movement will be the same as “PattenB1”. Please refer to wave on “Operating Pattern” screen below.



<Pattern C: Twice, square wave + Standstill>

This pattern is “Pattern A” which is performed twice, consecutively. On first and second movement, each parameter, you can set. Please refer to wave on “Operating Pattern” screen below.



3.2. Setting stretch and relax parameter

You can set parameter on “Operating Pattern setting” screen.

Each “Operating Pattern setting” screen is not same on each pattern. But standard parameters is two parameters below.

(1) Stretch ratio

(2) Stretch and relax speed (time)

<Setting Stretch ratio>

Please touch the white part next to “1=” on “Operating Pattern setting” screen. After, “Key pad” will be displayed on the screen. You can enter “Stretch ratio”. Parameter which you can enter is from 1% to 20%.

Stretch ratio 100% is 20mm,

Stretch ratio 1=1% ---------- Stretch length is 0.2mm.

Stretch ratio=20% ---------- Stretch length is 4.0mm.

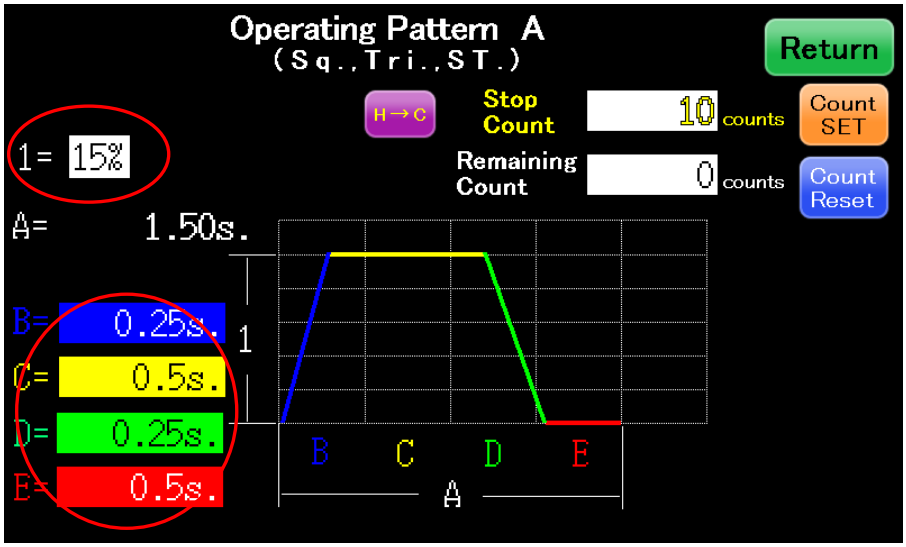
< Setting Stretch and relax speed (time)>

Here, we explain about Stretch and relax speed (time), on a case of “pattern A”.

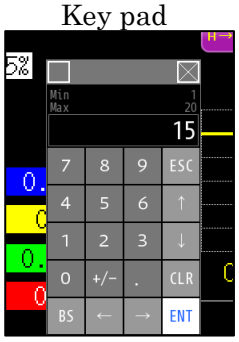
Please touch the white part next to each parameters (B, C, D, E) on “Operating Pattern setting” screen. After, “Key pad” will be displayed on the screen. Please refer to table1. about numbers which you can enter, and contents of each parameter.

Tabel1. Each parameter on “Pattern A”

Parameter sign	Contents	Input area
A=	One cycle time	Cannot input data
B=	Stretch movement time to specified stretch ratio (length)	0.25 --- 30.00sec.
C=	Standstill time on stretch	0.0 sec. --- 86400.0sec.(24h.)
D=	Relax movement time to non-stretch initial position	0.25 --- 30.00sec.
E=	Standstill time on relax (non-stretch initial position)	0.0 sec. --- 86400.0sec.(24h.)



Key pad



Other patterns, almost the same as “Pattern A”, but parameter contents of each Pattern is different. Please refer to below tables.

Table2. Each parameter on “Pattern B1”

Parameter sign	Contents	Input area
A=	One cycle time	Cannot input data
B=	Stretch and relax movement time(1/frequency)	0.5 --- 60.0sec.

Table3. Each parameter on “Pattern B2”

Parameter sign	Contents	Input area
A=	One cycle time	Cannot input data
B=	Stretch movement time to specified stretch ratio (length)	0.25 --- 30.00sec.
C=	Retention time on stretch	0.0 sec. --- 86400.0sec.(24h.)
D=	Relax movement time to non-stretch initial position	0.25 --- 30.00sec.
E=	Standstill time on relax (non-stretch initial position)	0.0 sec. --- 86400.0sec.(24h.)

Table4. Each parameter on “Pattern C”

Parameter sign	Contents	Input area
A=	One cycle time	Cannot input data
B=	(First time) Stretch movement time to specified stretch ratio (length)	0.25 --- 30.00sec.
C=	(First time) Standstill time on stretch	0.0 sec. --- 86400.0sec.(24h.)

D=	(First time) Relax movement time to non-stretch initial position	0.25 --- 30.00sec.
E=	(First time) Standstill time on relax (non-stretch initial position)	0.0 sec. --- 86400.0sec.(24h.)
F=	(Second time) Stretch movement time to specified stretch ratio (length)	0.25 --- 30.00sec.
G=	(Second time) Standstill time on stretch	0.0 sec. --- 86400.0sec.(24h.)
H=	(Second time) Relax movement time to non-stretch initial position	0.25 --- 30.00sec.
I=	(Second time) Standatill time on relax (non-stretch initial position)	0.0 sec. --- 86400.0sec.(24h.)

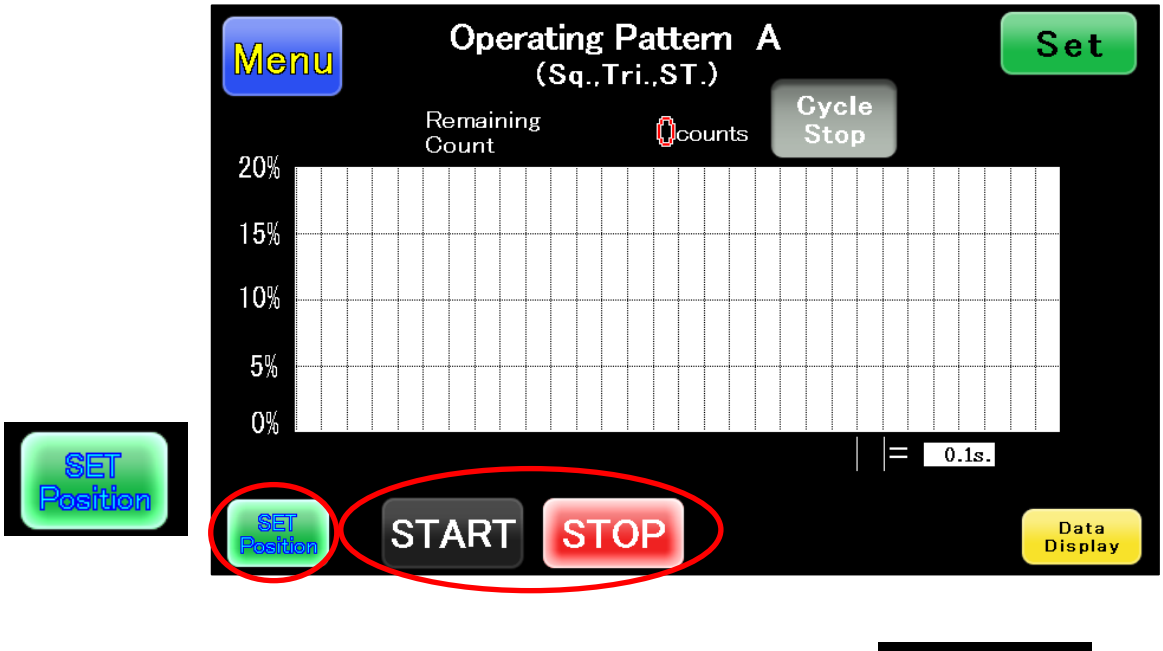
3.3. Operating to start and stop

<Start operation>

1.Chose which pattern of stretch, on “main menu” screen.

Display will go to “Operating Pattern” screen, you had chosen.

On “Operating Pattern” screen, when equipment stop, “START” button is flashing and “STOP” button is lighting. And, “SET Position” is displayed and its around area lights. (“SET Position” indicates that “stretch and relax point (bar)” is located on origin.).



2.Push “START” button on “Operating Pattern” screen.

After operation, Stretch and relax movement will start.

On stretch and relax movement, “START” button is lighting and “STOP” button lighting off.

And, displayed “NO SET Position” and not light its around area. (“NO SET Position” indicates that “stretch and relax point (bar)” is not located on origin.).

(Attention) On stretch and relax movement(displayed “NO SET Position”), you cannot change from “Operating Pattern” screen to “ Main menu” screen (This is interlock).

<Stop operation>

Push “STOP” button on “Operating Pattern” screen.

Stretch and relax movement will stop completely on the end of Stretch and relax cycle.

From pushing “STOP” button to stretch and relax movement cycle finishes, “START” button is lighting and “STOP” button is flashing.

(Attention) On this time, machine is moving, please attention!!

“START” button is flashing and “STOP” button is lighting, when machine completely stop. And, “SET Position” is displayed and light same its around area. (“SET Position” indicates that “stretch and relax point (bar)” is located on origin.).

<Forced Stop>

“Forced Stop” operation is to press and hold “STOP” button for 3 seconds and more, on stretching and relaxing movement.

After that operation, screen below will be shown on “Main display”.

“Main menu” screen is displayed, when “Forced Stop” is finished, completely

Press and hold
for 3 seconds and more.

“Forced Stop” will be used if you stop stretch and relax movement immediately.

“Stop operation” is the cycle stop (after finishing stretch, relax and standstill, Machine stop.).

“Forced Stop” can stop machine immediately, and “Stretch and relax point(bar)” move to “set position”.

(Attention) In that time, machine conducts returning to origin position.
Please conduct operation of “1.5. Switch on and check initial position (original point)” after “Forced Stop” operation. Because of origin return setting “Chamber holder” on “Operation unit”, there is a possibility that the origin position may change.

4. “Cycle Stop system” mode

“Cycle Stop system” mode makes “ShellPa Pro” stop, automatically, on setting counts of Stretch and relax cycle.

4.1. "Cycle Stop system" mode Setting

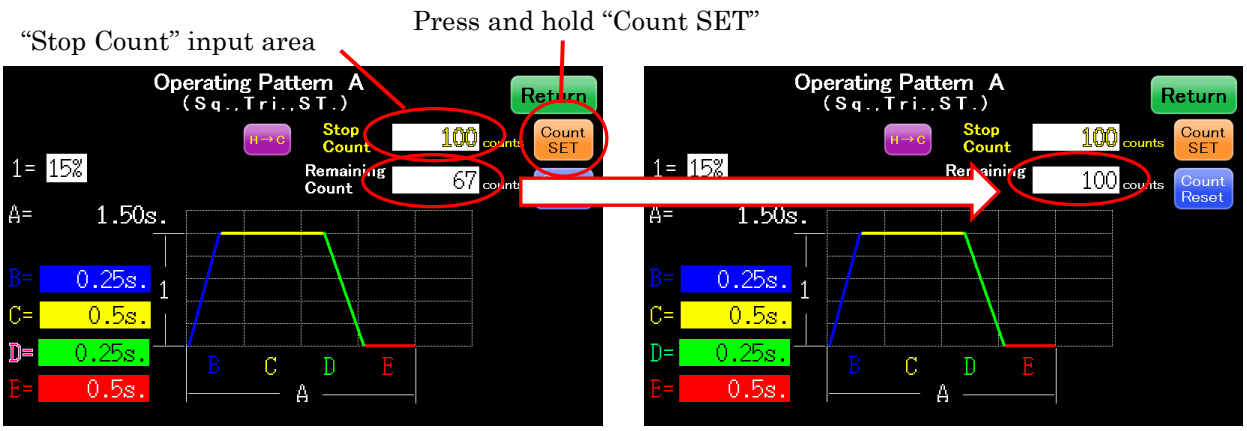
On “Main menu” screen, please set off “Stretch-Stop system”, because “Cycle Stop system” and “Stretch-stop system” cannot be used at the same time.

OFF

★ ----- Next, go to “Operating Pattern setting” screen on each pattern, you select.

Please touch the white part next to “Stop Count” on “Operating Pattern setting” screen. After, “Key pad” will be displayed on the same screen. You can enter number, of stretch and relax cycle until stop, to “Stop Count”. Parameter “Stop Count” which you can enter, is from 1 to 9,999,999. (Please attention, at this time, still number of “Cycle Stop” is not set.)

Next, ”Count SET”, please press and hold, for 3 seconds and more. Number of “Stop Count” will be set to “Remaining Count”. (On this time, finished setting number of “Cycle Stop”.)



4.2. "Cycle Stop system" mode operation

<"Cycle Stop system" mode "START" and "STOP" operation>

You can set "Cycle Stop system" mode ON and OFF on "Operating Pattern" screen.

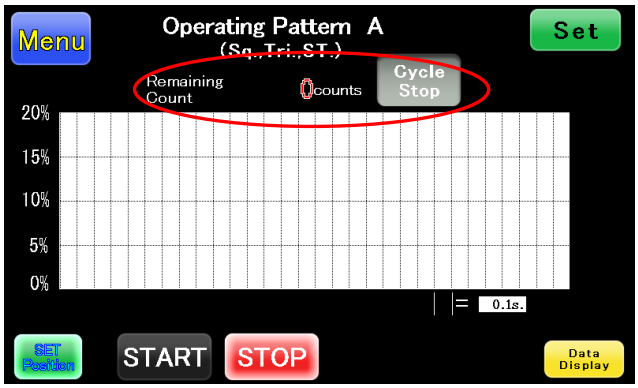
Please touch "Cycle Stop" on "Operating Pattern" screen. You can change "Cycle Stop system" mode ON and OFF, touching "Cycle Stop" on "Operating Pattern" screen.

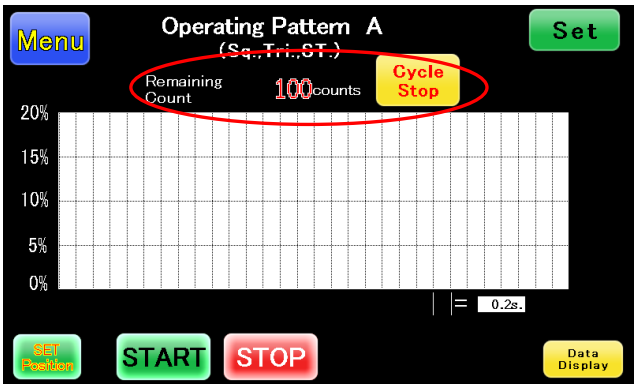
When "Cycle Stop" on "Operating Pattern" screen, is lighting (Colored with yellow), "Cycle Stop system" set ON.

That time ("Cycle Stop system" set ON.), number of stretch and relax cycle until stop, as "Remaining Count" on "Operating Pattern" screen, is displayed.("Remaining Count" on "Operating Pattern setting" screen, is displayed in "Remaining Count" on "Operating Pattern" screen.)

(When "Cycle Stop" on "Operating Pattern" screen, is not light, "Cycle Stop system" set OFF. That time ("Cycle Stop system" set OFF.), That "Remaining Count" is "0", is displayed.)

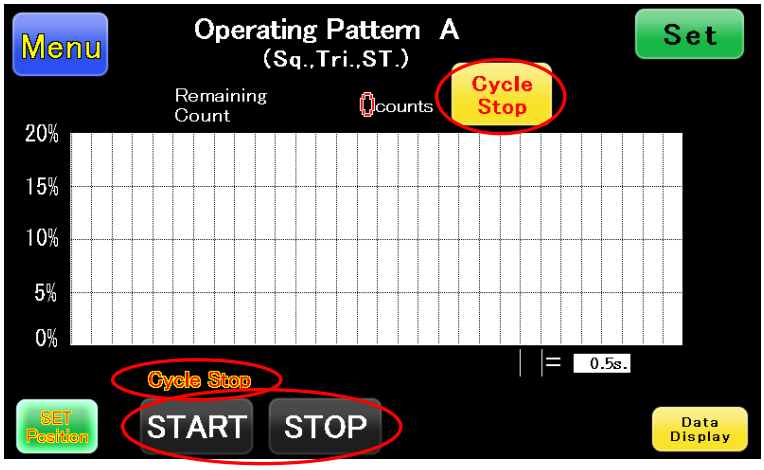
"START" and "STOP" operation is identical with a standard "START" and "STOP" operation. Please refer to "3.3. Operating to start and stop". After starting, "Remaining Count" will decrease, every time cycle is over.





<Operation on counting up "Cycle Stop system">

Counting up "Cycle Stop system" (Remaining Count" is "0"), "START" and "STOP" not light, and "Cycle Stop" button flash, and "Cycle Stop" sign is displayed, on "Operating Pattern" screen.



Please set “Cycle Stop” off (touch “Cycle Stop”).

If you operate “Cycle stop” mode again, please carry out ★(referring to “4.1. “Cycle Stop system” mode Setting” on the previous page).

5. “Stretch-Stop system “mode

“Stretch-Stop system” mode makes Stretch and relax movement start and stop, automatically, and it repeat, according to setting parameters.

5.1. "Stretch-Stop system" mode ON/OFF

By touching ”Stretch-Stop System ON/OFF” on “Main menu” screen, you can change “Stretch-Stop system“ mode ON/OFF,.

Stretch-Stop System ON: ”Stretch-Stop System ON” sign and button light on.

Stretch-Stop System OFF: ”Stretch-Stop System OFF” sign and button light off.

5.2. "Stretch-Stop system" mode Setting

You can set parameter on “Stretch-Stop system setting” screen.

Please touch the yellow part next to each parameters (“Stretch time”, ”Stop time”, ”Number of Sets”) on “Operating Pattern setting” screen. After, “Key pad” will be displayed on that screen. Please refer to Table5. about numbers which you can enter, and contents of each parameter.

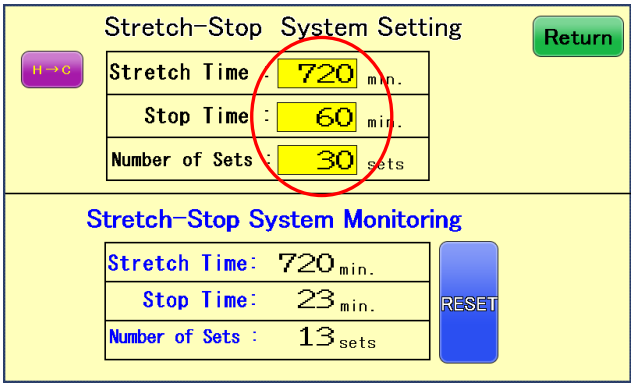


Table5. Each parameter on “Stretch-Stop system setting”

Parameter sign	Contents	Input area
Stretch Time	Stretch, relax and standstill time	1 --- 1,440min.

	(State of starting stretch and relax pattern)	
Stop Time	Stop time	1 --- 1,440min.
Number of Sets	Number of repeated “Stretch Time + Stop Time”	1 --- 100sets

conducted on order below,

First: Stretch Time
Second: Stop Time
} 1 set

First and Second repeat on set “Number of Sets”. Please refer to “Fig. 1 number of set on “Stretch-Stop system”.

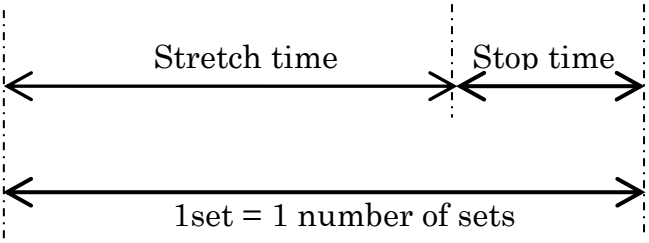
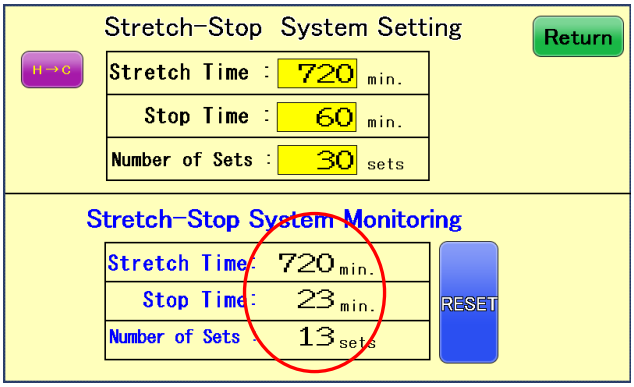
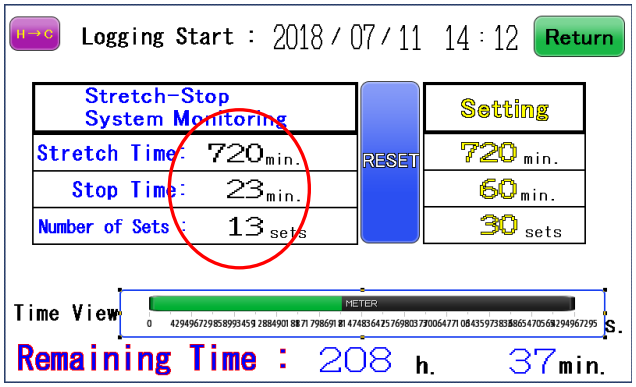


Fig. 1 number of set on “Stretch-Stop system”

5.3. "Stretch-Stop system" mode Monitoring

You can check elapsed time on “Stretch-Stop system Setting” or “Stretch-Stop system Monitoring”. “Number of Sets” is integrated, but “Stretch Time” and ”Stop Time” reset every finished 1 set (1 set = “Stretch Time” + ”Stop Time”)



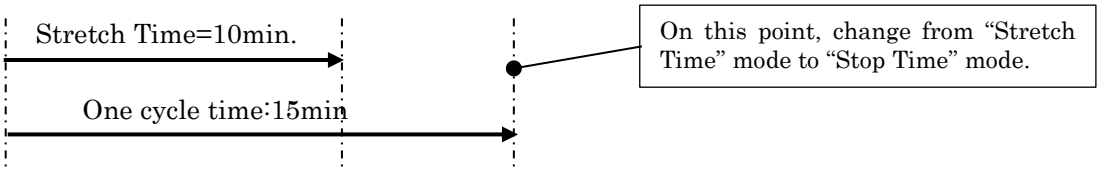


After finishing one cycle (stretch and relax movement), “Stretch-Stop system” changes from “Stretch Time” mode to “Stop Time” mode. Therefore, there is possibility to take “Stretch Time” take time longer than setting time (refer to example). And, “Remaining Time” is overview time (“Remaining Time” is calculated time, It is updated (recalculated) every time one set finishes.)

<Example>

Setting “Stretch time”: 10min.

One cycle time (Parameter A of “Operating Parameter setting”): 15min.(900sec.)



Setting “Stretch time” is 10min., but actually, it will change from “Stretch Time” mode to “Stop Time” mode, 15min. (is the time when one cycle ends.) later.

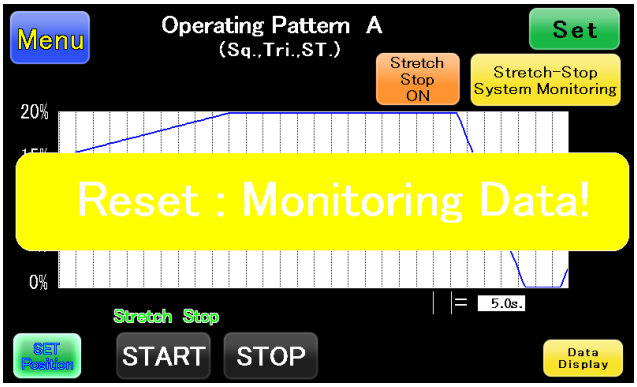
5.4. “Stretch-Stop system “mode operation

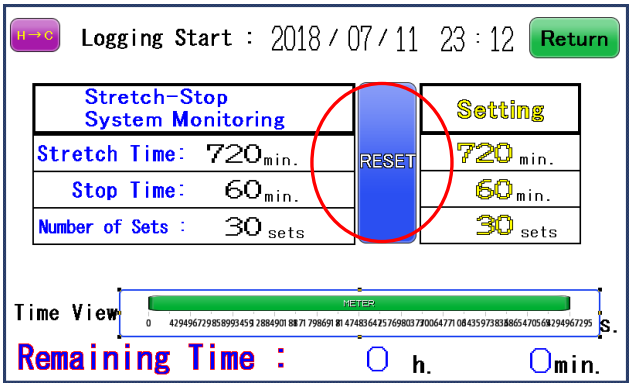
<"Stretch-Stop system" mode “START” and ”STOP” operation>

“START” and ”STOP” operation is identical with a standard “START” and ”STOP” operation. Please refer to “3.3. Operating to start and stop”.

<Operation on counting up “Stretch-Stop system”>

Counting up “Stretch-Stop system” (“Number of sets” is full), below screen will be displayed on “Operating Pattern” screen, and “RESET” button on “Stretch-Stop system Monitoring” screen and, is flashing. And Stretch and relax movement stops.





At this time, “START” and ”STOP” not light and cannot be used. “Stretch Stop” sign is displayed and flashes.

Please press and hold “RESET” button on “Stretch-Stop system Monitoring” screen, for 3 seconds and more. Monitoring counter of “Stretch-Stop system” will set “0” on each parameter.

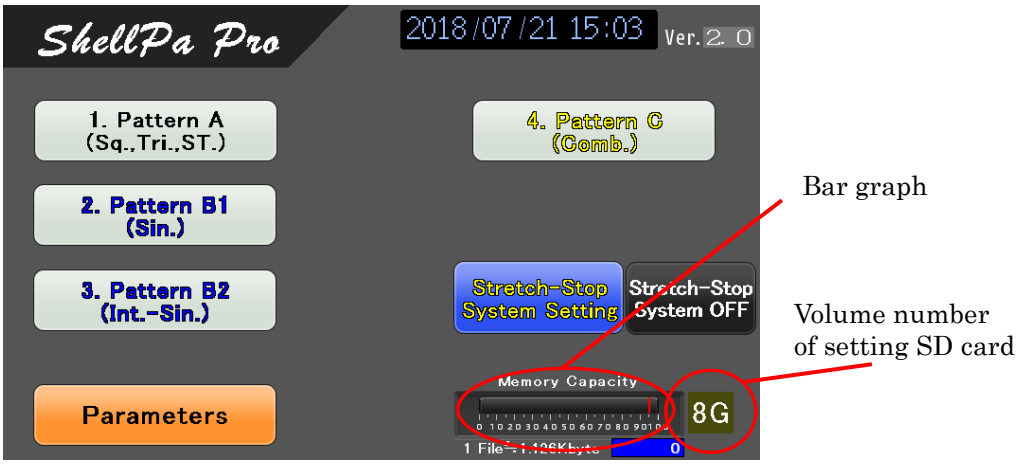
If you carry out “Stretch-Stop system” mode again, please try again from the first of this chapter.

6. Data output to USB, and viewing images on a computer (Data Display screen)

"ShellPa Pro" can store data of stretch and relax movement in USB, and on PC, you can check.

6.1. Set SD card

If SD card and parameters of setting SD card has been set, Bar graph and volume number of setting SD card is displayed on "Main menu" screen. (If SD card and parameters is not set, "Memory No Set" or "NO SET" will be displayed.). Please check.



(Attention: Ordinary, we insert and set 8Gbyte SD card, and shipped. Therefore, below work, you generally not need.)

(Attention: Changing parameters of SD card, or taking out and in SD card, clear (reset) bar graph, because this bar graph directly does not check SD card.)

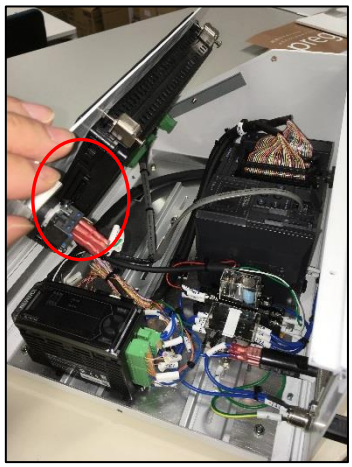
<SD card insert>

On case of no SD card, "Memory No Set" is displayed on "Main menu" screen.

SD card inserts place, please refer to photo below on "Control unit".

You must open "Control unit".

Please attention!!, Each control equipment, Connecter, and cable…



< Setting parameters of setting SD card>

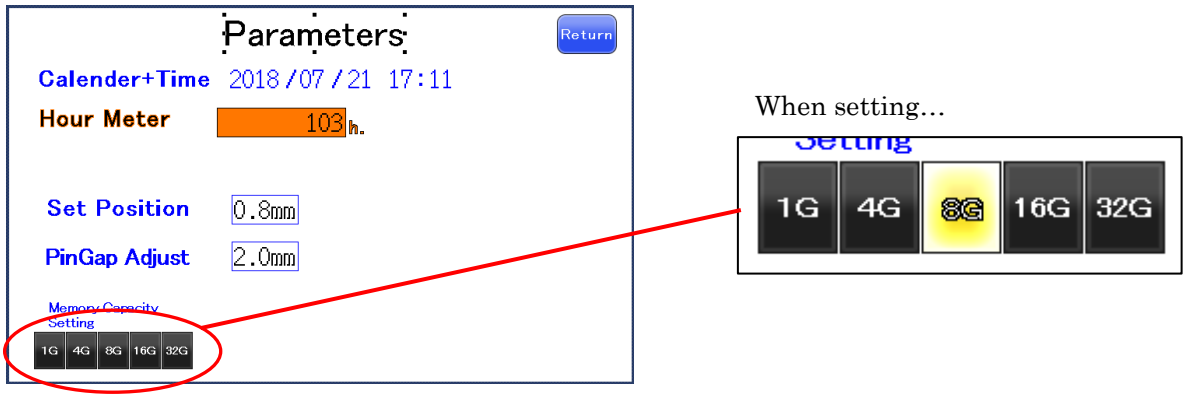
Not setting Volume number parameters of setting SD card, "NO SET" is displayed on "Main menu" screen.

You can set volume number parameters of setting SD card on "Parameters" screen.

On "Parameters" screen, please check "Memory Capacity Setting".

Please press and hold volume number button which volume of inserting SD card is equal to , on "Stretch-Stop system Monitoring" screen, for 3 seconds and more.

When setting, volume number button is colored with yellow.

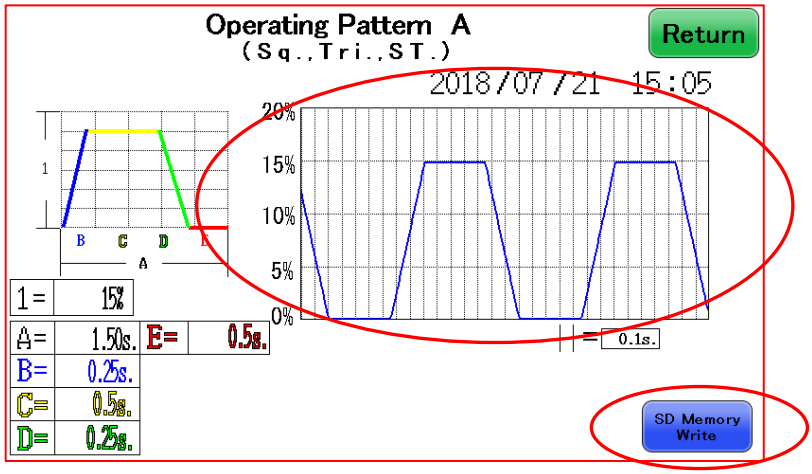


6.2. Store data of stretch and relax movement in USB

You must conduct, First, Store Data in SD card. Second, after it, change over from SD card to USB.

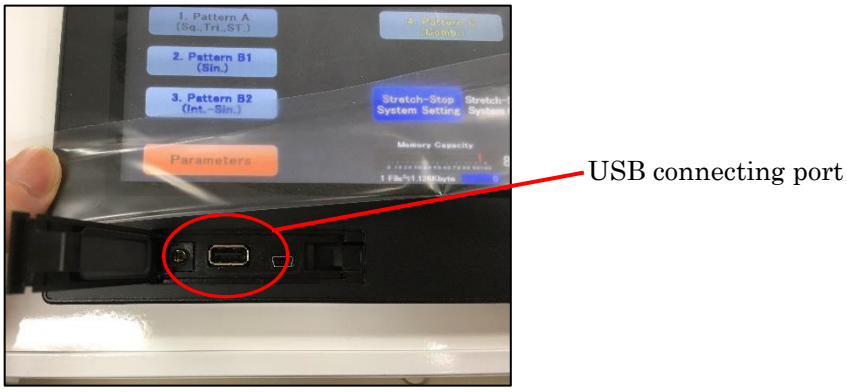
First: Store Data in SD card.

Please Check Stretch and relax wave data displayed on “Operating Pattern data output” screen. If O.K., Please press and hold, for 3 seconds and more, “SD Memory Write” button on “Operating Pattern data output”. Data is written on SD card.



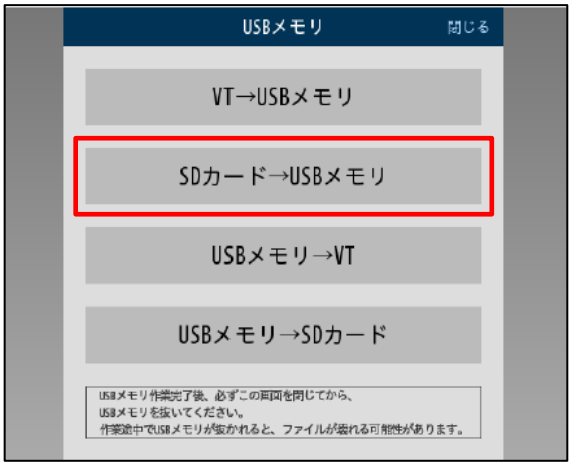
Second: Store change over from SD card to USB.

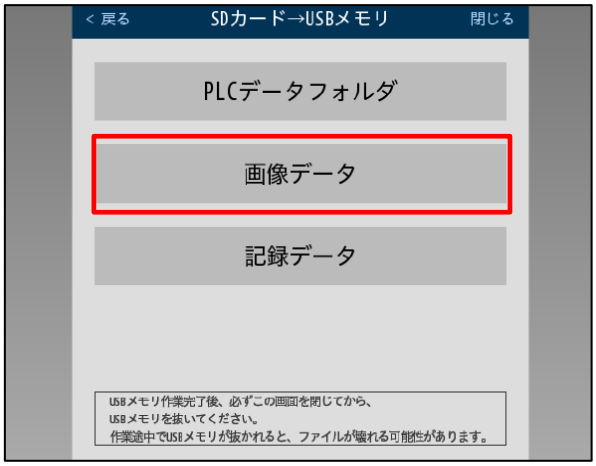
Please insert USB to connecting port.



After it, automatically, the display change to “USB memory”.

Select “SD card → USB memory” on “USB memory” screen, the display change from “USB memory” to “SD card → USB memory” screen.



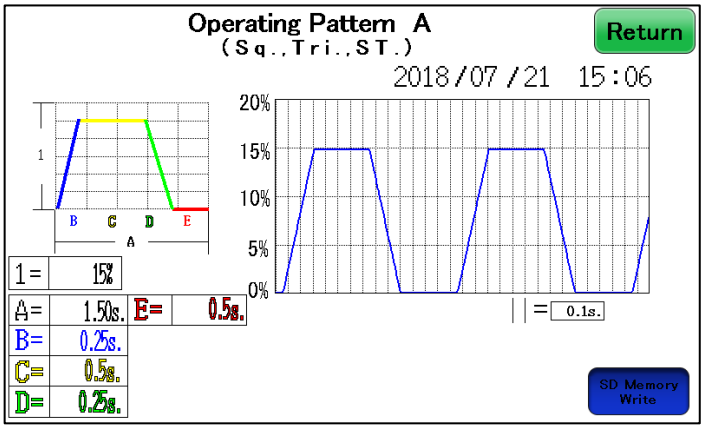


Next, Select “Display data” on “SD card → USB memory” screen. Started to write data to USB, and “Not pull out USB” is displayed.

When writing data on USB is finished, “Data copy is finished” is displayed.

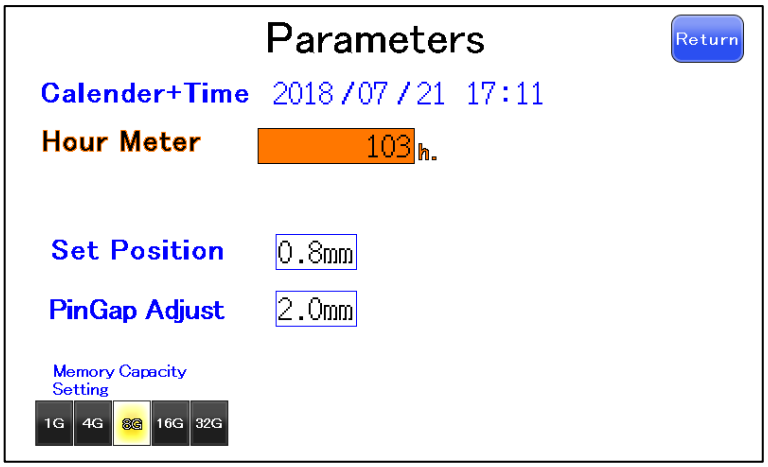
After it, please pull out USB, automatically, change to “Operating pattern” screen.

On your PC, you can check and view Stretch and relax wave data in bitmap format.



ON YOUR PC

7. “Parameters” screen



7.1. ”Calender + Time”

You can change and set, calender and time.

On “Parameters” screen, please touch Number which you want to change, after, “Key pad” will be displayed on the screen. Please change number.

7.2. ”Hour Meter”

You can check total operation time on this machine.

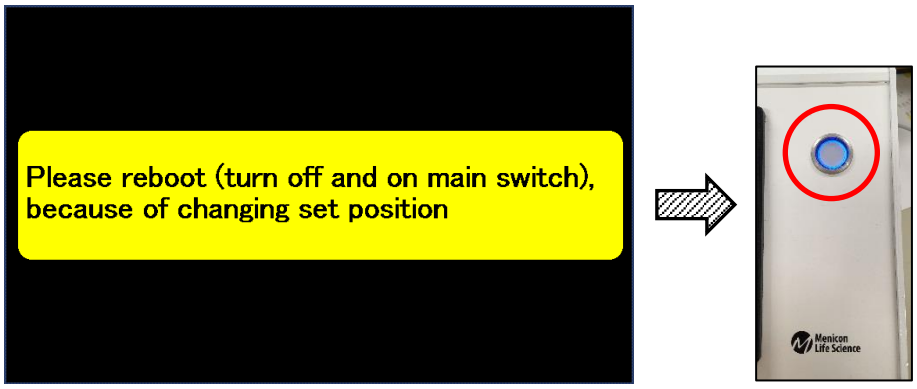
But you cannot change this number, check only.

7.3. ”Set Position” and “Pin Gap Adjust”

These parameters mean,

“Set Position”: Position to set “Chamber holder” parts

(Attention: When you change “Set Position”, Machine require turn off and on. Please conduct it.)



“Pin Gap Adjust”: Position that machine consider as Stretch ratio 0%.

Please understand below movement.

==========

--- Main switch on: “Origin(0.0mm)” → “Set Position” (Stationary)

--- Push “Start”:

“Set Position” → “Pin Gap Adjust” --- “Pin Gap Adjust + Stretch ratio position”

(Stretch and relax movement)

--- Push “Stop”: “Pin Gap Adjust” → “Set Position” (Stationary)

(Attention: Machine is always located on “Set Position”, in stop state.)

==========

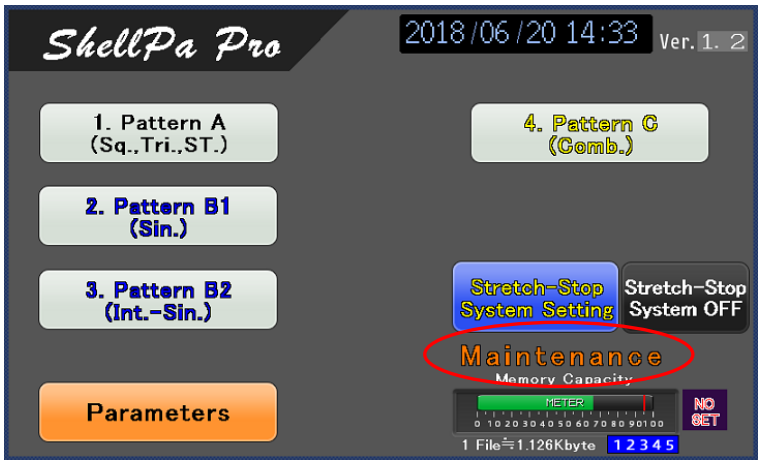
8. “Maintenance” and “Overhaul” alarm

“ShellPa Pro” will inform you that operating time reaches a certain time. Please check below.

(Attention: These alarms are just information. Therefore, on these alarms, equipment can operate, ordinary.)

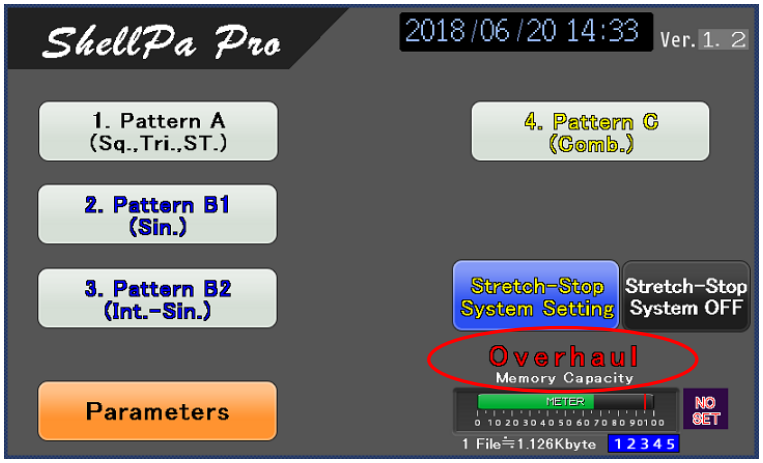
<Maintenance: When operating time reaches 2,000 hours.>

“Maintenance” alarm flashes on “Main menu” screen, when operating time reaches 2,000 hours.



<Overhaul: When operating time reaches 8,000 hours.>

“Overhaul” alarm flashes on “Main menu”, when operating time reaches 8,000 hours.

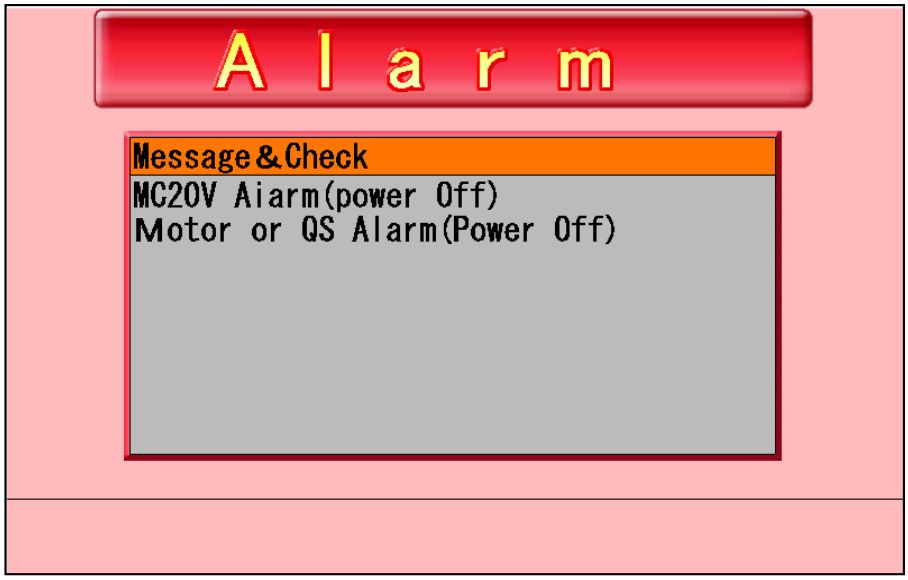


Attention: Users cannot reset these alarms. Please contact us (refer to “12. Vendor information”), if you want to reset these alarms.

9. “Alarm” and Trouble shooting

9.1. “Alarm”

When abnormalities of equipment happen, below “Alarm” screen is displayed.



detail Alarm contents, Please check below table6. Alarm sign.

Table6. Alarm sign

Alarm sign	Contents	Memo
MC20V Alarm (Power Off)	Internal calculation error (Controller equipment system have abnormality.)	Please turn off and on (It means “reset” operation). If alarm is not resolved, Please contact us.
Motor or QS Alarm (Power Off)	Abnormality of Motor system.	Please turn off and on (It means “reset” operation). If alarm is not resolved, Please contact us.

9.2. Trouble shooting

If you have any problems, please check below Table7.

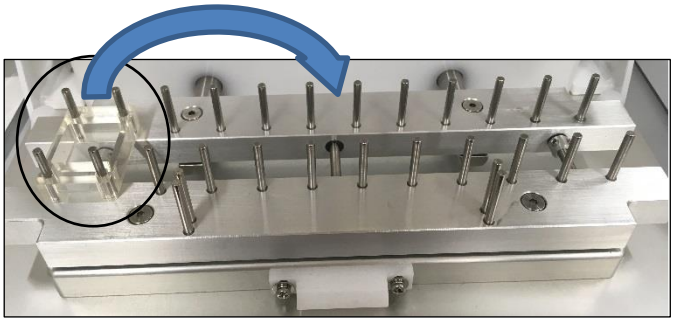
Table7. Trouble shooting

Trouble	Check	Memo
Electricity is not supplied	Check connecting “Power supply adaptor” , “Power supply cable” and “Control unit”.	---
Operation unit do not move.	Check connecting “Connecting cable” and “Control unit”.	---
Cannot operate to change “Operating pattern” screen to “Main menu” screen.	Check machine stops, or “STOP” button lighting off on “Operating pattern” screen	You can change “Operating pattern” screen to “Main menu” screen, when Stretch and relax movement completely stops. (It is interlock.)
Stretch and relax position changed.	Had you already conducted operation of return to origin , removing “Stretch chamber holder”. Please conduct operation of return origin, removing “Stretch chamber holder”. (refer to “1.4. Switch on and check initial position (original point)”)	On setting “Stretch chamber holder”, operating return to origin, there is possibility to change the position of origin. And, It’s same, in case of operating “Forced stop”. (refer to <Forced stop> in “3.3. Operating to start and stop”)

10. Attention !!!

Do not set and start stretch motion in below setting. There is a possibility of being broken for Machine.

Would you please set ”Stretch chamber” on “Stretch chamber holder”, well-balanced.



11. Specification

Table8. Specification

Product Name	ShellPa Pro
Model type	SSMP
Dimensions (Approx.)	“Operation unit” : W285 mm × D300mm × H120mm “Control unit” : W321 mm × D270mm × H155 “Connecting cable” length : 2.3m
Mass (Approx.)	“Operation unit” : 6.2kg, ”Control unit”: 4.3kg
Power requirement	100 to 240V , 47 to 63Hz
Output Voltage	D.C.24V , 2.5A (60W)
Stretch ratio	Input ratio range 1 to 20%, possible to input 1% unit.
Stretch and retention time	Input “stretch/relax time” 0.25 to 30.00s., possible to input 0.01s, unit Input “retention time” : 0.0 to 24h (0.0 to 86,400.0s.) ,possible to input 0.1s, unit.

12. Vendor information

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Web: https://www.menicon-lifescience.com/english/

Appendix 1

1. Chamber coating procedure

(1) Coating with fibronectin

1) Dilute fibronectin with PBS to the concentration of 2 to 20 μg/mL.
2) Set a stretch chamber SC4Dea into a dish, add 1 to 1.5 mL of the diluted fibronectin solution 1) to the chamber. Gently tap the dish to completely cover the bottom of the chamber.
3) Incubate the dishes for 30 minutes to 4 hours at 37°C.
(If the coating is not sufficient, please extent the incubation time.)
4) Remove the coating solution and wash 2-3 times with PBS or serum free culture medium. Seed the cells to start culture.

(2) Coating with collagen

1) Prepare dilute hydrochloric acid (pH=3.1mM) and autoclave.
2) Dilute collagen (type I or type 4) with dilute hydrochloric acid 1).
3) Set a stretch chamber SC4Dea into a dish, add 1 to 1.5 mL of the diluted collagen solution 2) to the chamber. Gently tap the dish to completely cover the bottom of the chamber.
4) Incubate the dishes for 30 minutes to 4 hours at 37°C.
(If the coating is not sufficient, please extent the incubation time.)
5) Remove the coating solution and wash 2-3 times with PBS or serum free culture medium. Seed the cells to start culture.

(3) Coating with laminin

1) Dilute laminin with PBS to the concentration of 1 μg/mL.
(Natural Mouse Laminin (Gibco #23017-015)
http://www.lifetechnologies.com/order/catalog/product/23017015)
2) Set a stretch chamber SC4Dea into a dish, add 1 to 1.5 mL of the diluted laminin solution 1) to the chamber. Gently tap the dish to completely cover the bottom of the chamber.
3) Incubate the dishes for 30 minutes to 4 hours at 37°C.
(If the coating is not sufficient, please extent the incubation time.)
4) Remove the coating solution and wash 2-3 times with PBS or serum free culture medium. Seed the cells to start culture.

(4) Coating with PDL

1) Dilute PDL with serum free medium to the concentration of 4 mg/mL for storage at -20°C.
(Poly-D-Lysine Hydrobromide, High Molecular Weight (BD #354210))
2) Prior to use, dilute the stock PDL solution to the final concentration of 50 μg/mL.

3) Set a stretch chamber SC4Dea into a dish, add 1 to 1.5 mL of the diluted PDL solution 2) to the chamber. Gently tap the dish to completely cover the bottom of the chamber.
4) Incubate the dishes for 1 to 4 hours at 37°C.
(If the coating is not sufficient, please extent the incubation time.)
5) Remove the coating solution and wash 2-3 times with PBS or serum free culture medium. Seed the cells to start culture.