

How to use SEC-SAXS [for SHIMADZU Nexera/Prominence-i] (2020.07.10)

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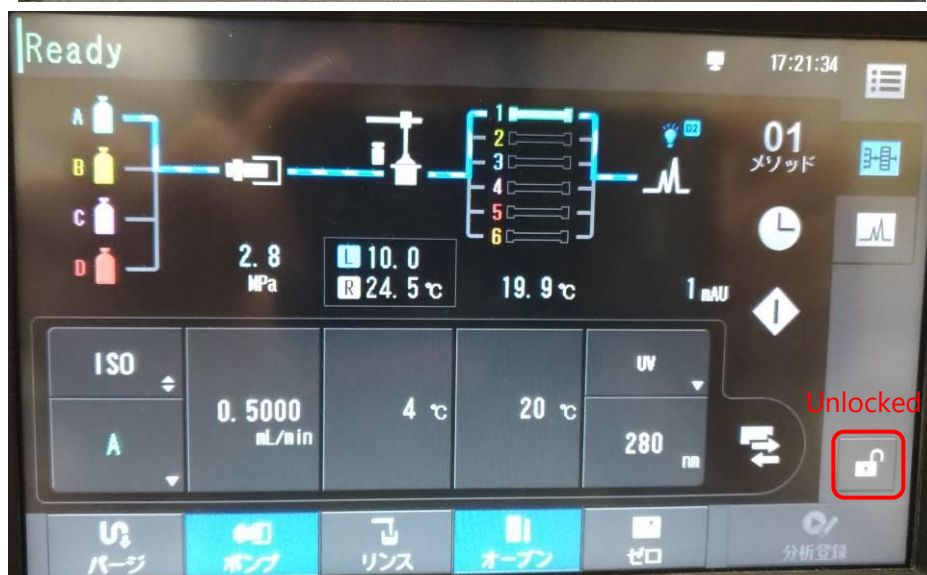
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0 Preparation of HPLC system

0-1

When Lab solutions (Control Software of HPLC) are launched on the PC, the operation from the front LCD panel of the HPLC is locked.

When you operate the HPLC from the front LCD panel for buffer exchange or other operations, touch the lock icon (1) on the front LCD panel and enter the password: 00000 (2) to unlock the HPLC.



0-2

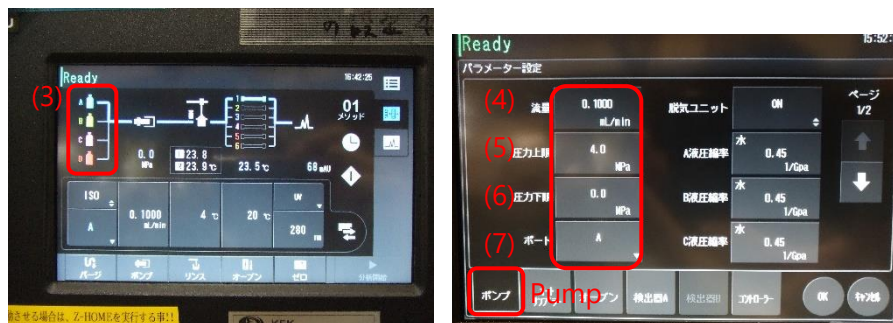
Touch the solvent bottle icon (3) on 分析メイン画面 [Analysis Main Screen] of the front LCD panel to open ポンプタブ [Pump tab] on [Parameter screen], and check the following parameters.

流量 [Flow rate] (4): Set same value at 1A-7

圧力上限 [Upper pressure limit] (5): 4.0 MPa (default: See the manual of the your SEC column)

圧力下限 [Lower pressure limit] (6): 0.0 MPa (default: See the manual of the your SEC column)

ポート [Port] (7): A



0-3

Open オートサンプラータブ [Auto Sampler] tab on [Parameter screen], and check the following parameters.

クーラーラックプレート L [Rack plate cooler L] (8): ON

クーラーラックプレート R [Rack plate cooler R] (9): OFF

サンプルクーラー温度 [Sample rack temperature] (10): 4°C (set the appropriate value)



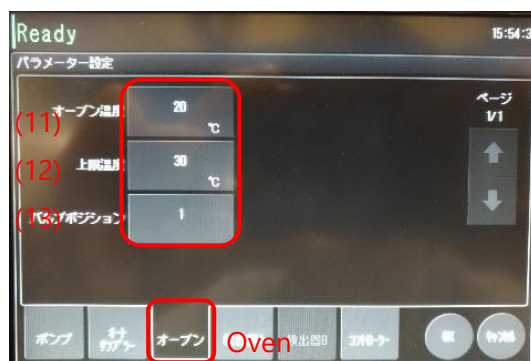
0-4

Open オープンタブ [Oven] tab on [Parameters screen], and check the following parameters.

オープン温度 [Column oven temperature]: 20°C (set appropriate value)

上限温度 [Upper temperature limit]: 30°C (when column oven temperature is set to 4~25°C)

バルブポジション [Valve position]: 1



0-5

Open 検出器 A タブ [detector A] tab on [Parameters screen], and check the following parameters.

波長 1 [Wavelength 1] (14): 280 nm

ランプ [Lamp] (15): D2

セル温調 [UV cell temperature control] (16): ON

セル温度 [UV cell temperature] (17): 30°C [fixed]



0-6

Touch [OK] icon (18). If they are not started, the Temp control of auto sampler and D2 lamp start. And the temp indicator of sample rack L and D2 lamp indicator [bulb icon] are change to light blue color.

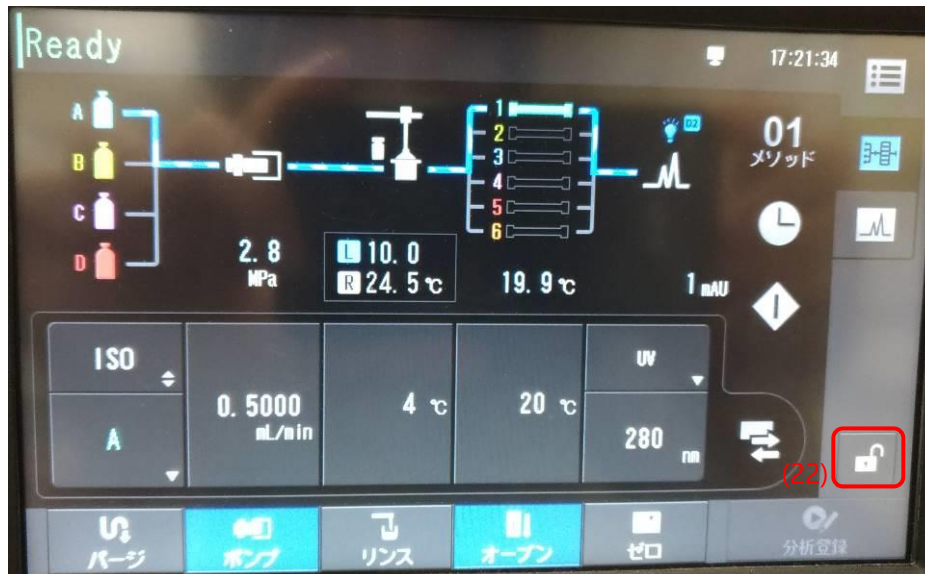
0-7

Touch ポンプ [pump] icon (19) to start flow (you can set the flow rate (20)), and touch オープン [oven] icon (21) to start the column oven (The icon turns light blue while it's working.)



0-8

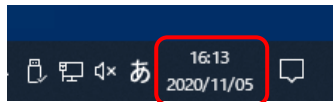
Touch the lock icon (22) to lock the HPLC.



0-9

Synchronization of PC clock (for HPLC control PC)

Check the time of HPLC control PC (remote desktop on BL control or Measurement PC) and the time of measurement PC. If they are same, proceed to 0-13. If they are out of sync, right-click on the clock in the bottom right corner of the remote desktop window PC and select the second line "日付と時刻の調整 (A)" [Adjust Date and Time].



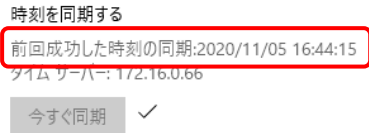
0-10

“時刻の同期” window come appear in the remote desktop. Please click [今すぐ同期] in “時刻を同期する”.

Time server of each beamline is as follows. Please click this button several time if you see the error. Finally, the time stamp of synchronization will be updated.

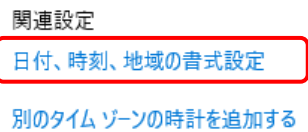
BL-10C: 172.16.0.66

BL-15A2: 172.16.0.1



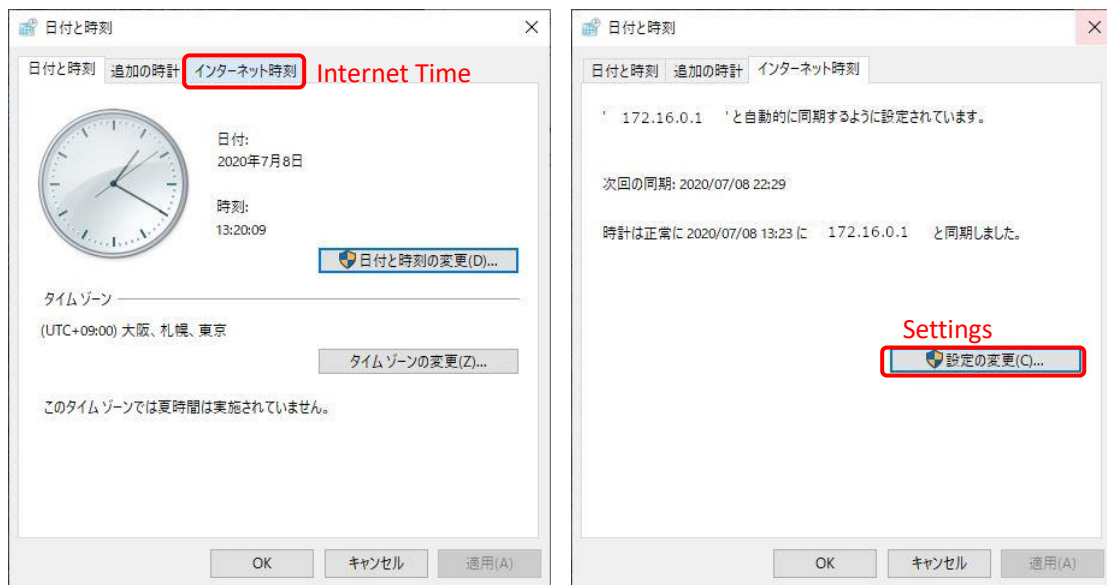
0-11

In the case that the combination between beamline and IP address of time server is different (For example, HPLC of BL-15A2 is used at BL-10C because of trouble.), click “別のタイムゾーンの時計を追加する” in the upper right side of this window.



0-12

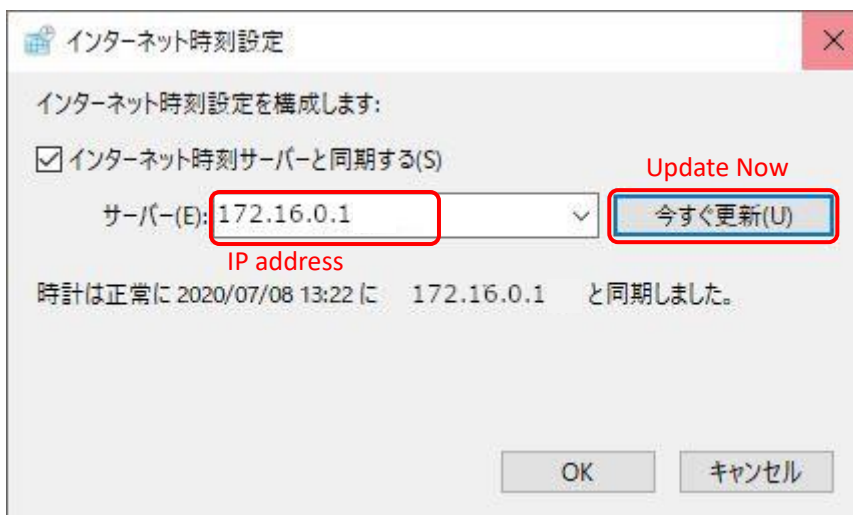
In “日付と時刻” [Date and Time] window, click on “インターネット時刻” [Internet Time] tab and then click “設定の変更” [Settings].



0-12

In “インターネット時刻設定” [Internet Time] tab, enter the IP address of time server for each beamline as shown below and click “今すぐ更新” [Update Now].

BL-10C	172.16.0.66
BL-15A2	172.16.0.1



0-13 Synchronization of PC clock (for Spectrometer control PC)

Check the time of spectrometer control PC (remote desktop on measurement PC) and the time of measurement PC. If they are same, proceed to next chapter.

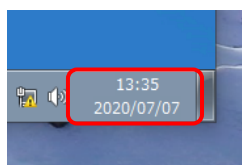
If they are out of sync,

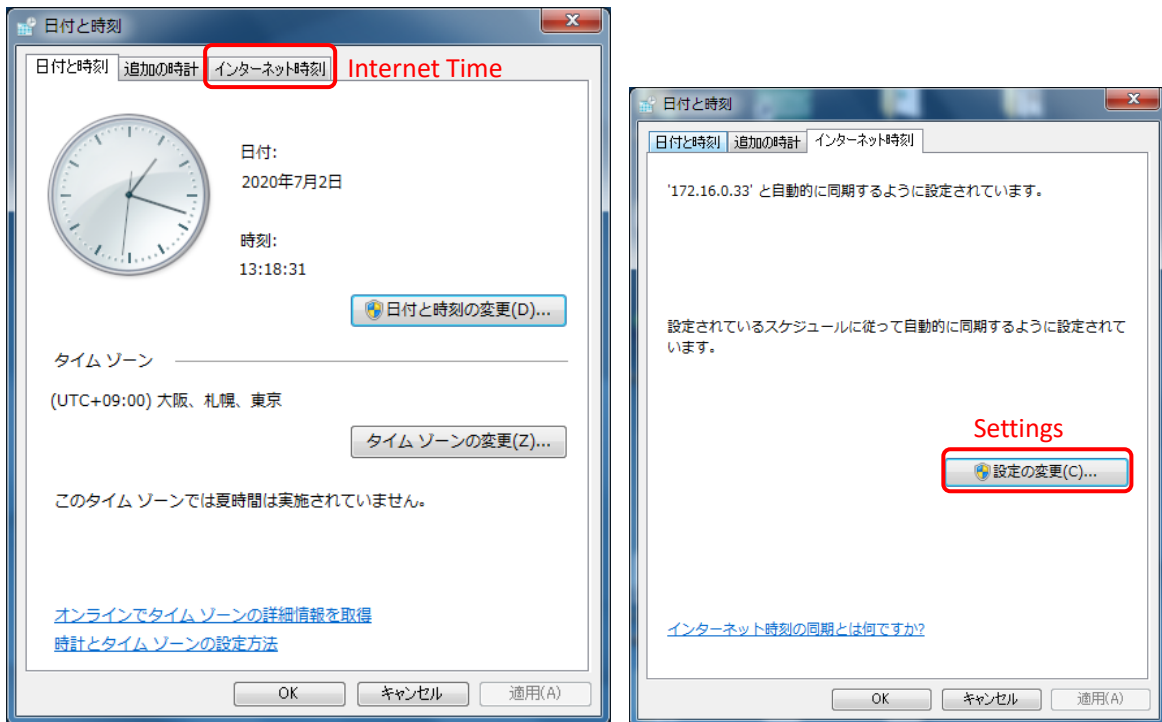
Firstly right-click on the clock in the bottom right corner of the desktop on spectrometer control PC (remote desktop on BL measurement PC).

Secondly select the second line "日付と時刻の調整 (A)" [Adjust Date and Time].

Thirdly click on "インターネット時刻" [Internet Time] tab in "日付と時刻"[Date and Time] window.

Finally click "設定の変更" [Settings].

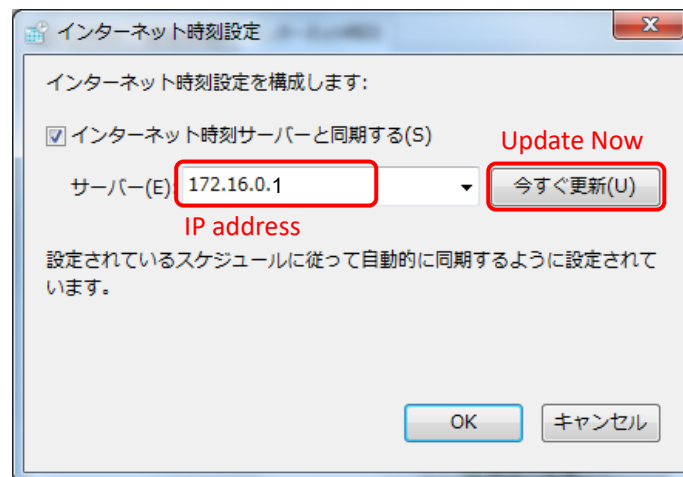




0-14

In “インターネット時刻設定” [Internet Time] tab, enter the IP address of time server for each beamline as shown below and click “今すぐ更新”[Update Now].

BL-10C	172.16.0.66
BL-15A2	172.16.0.1



1 Exchanging buffer / Equilibration of SEC column

1A Using PF's SEC column (The SEC column was already connected to HPLC system).

1B Using your SEC column.

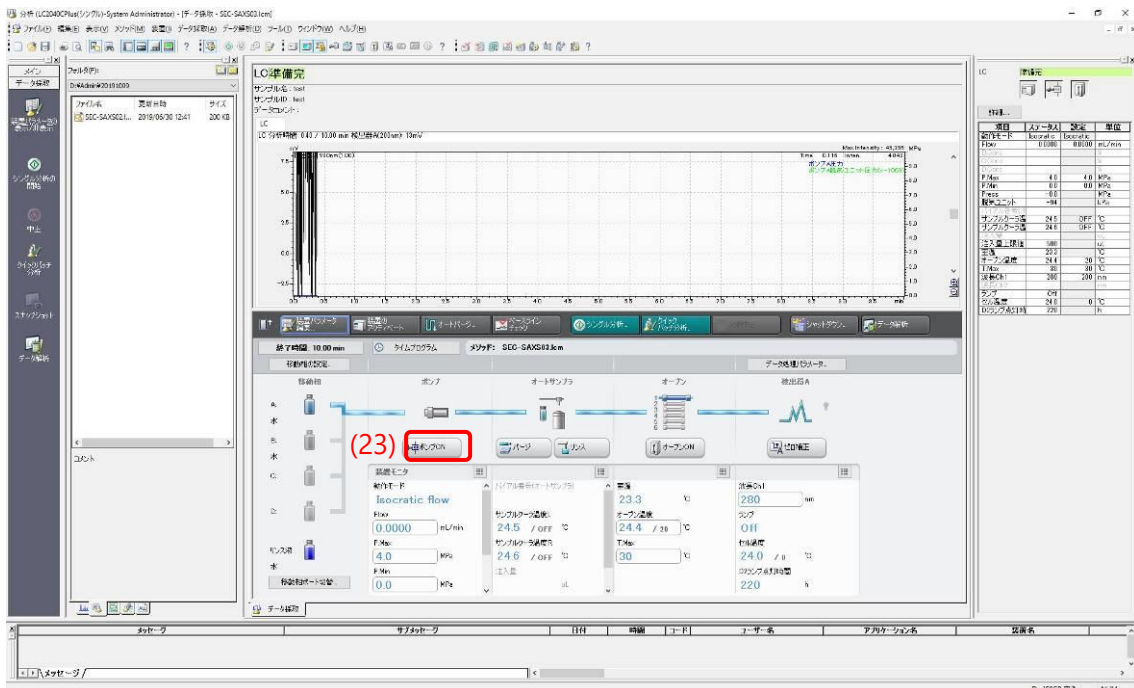
1A Using PF's SEC column (The SEC column was already connected to HPLC system).

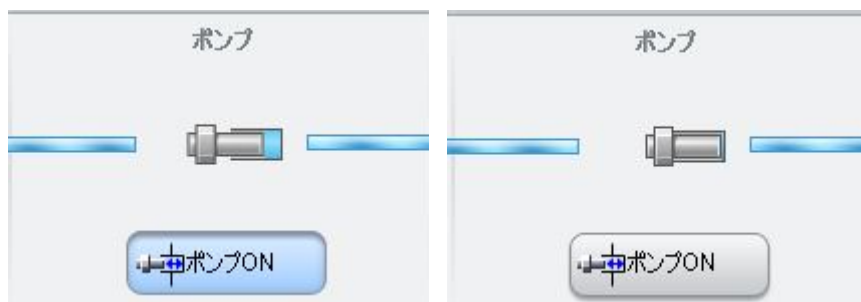
At the beginning of your BT, the BL staff set up the system as described below.

- HPLC system, UV spectrophotometer and PILATUS have been connected and started.
- The SEC column has been connected to HPLC system and equilibrated with MilliQ (or other buffer). The flow rate is set to low [for example 0.05 ml/min]. (Buffer should be filtered and degassed. Degassing your buffer @Biophysics & Molecular physiology Lab. of PF is recommended.)
- Lab Solution (HPLC control software) can be operated by remote desktop on BL control PC.

1A-1

Click **ポンプ ON** [Pump ON] icon (23) in **データ採取タブ** [Data Collection] tab at the lower middle of the Lab Solution screen to stop the pump. [Operating: light blue, stopped: gray]





1A-2

Wait until the pressure (Press) status (24) on the right side of the screen reaches 0M Pa.

項目	ステータス	設定	単位
動作モード	Isocratic	Isocratic	
Flow	0.0000	0.0000	mL/min
B.Conc			%
C.Conc			%
D.Conc			%
P.Max	4.0	4.0	MPa
P.Min	0.0	0.0	MPa
Press	-0.0	(24)	MPa
脱気ユニット	-94		kPa
ハイアル番号(オ)			
サンプルクーラ温	24.5	OFF	°C
サンプルクーラ温	24.6	OFF	°C
注入量			uL
注入量上限値	500		uL
室温	23.3		°C
オープン温度	24.4	20	°C
T.Max	30	30	°C
波長Ch1	280	280	nm
波長Ch2			nm
ランプ	Off		
セル温度	24.0	0	°C
D2ランプ点灯時	220		h

1A-3

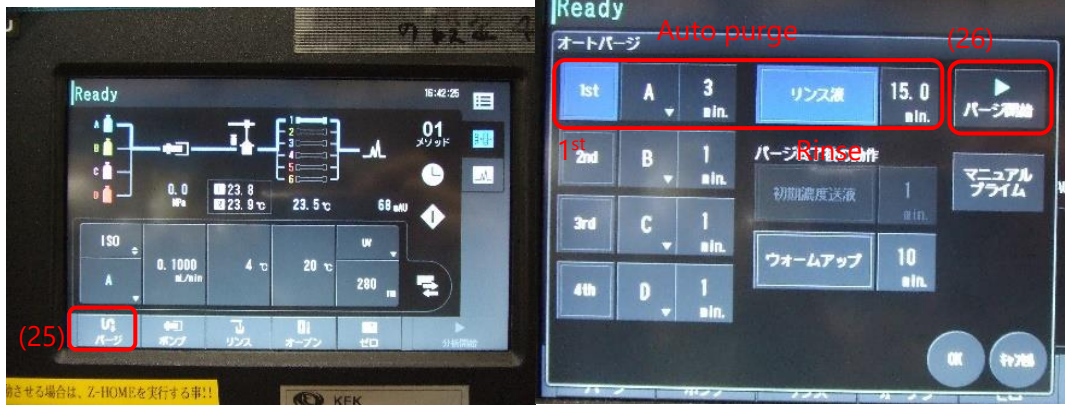
Place your buffer bottle on the tray of the HPLC system. Put Solvent A line (cyan labelled) into your buffer bottle, and cover the spout of the bottle with parafilm.

1A-4

Unlock HPLC control from the front LCD panel (See 0-1), and touch パージ [Purge] icon (25) on the [Analysis Main Screen] to open オートパージ [Auto Purge] screen.

Turn on [1st] and リンス液 [Rinse] (light blue color indicates 'ON').

Enter purge time ([1st]: A, 3min, リンス液 [Rinse]: 15min).



1A-5

Touch **パージ開始** [Start Purge] icon (26) to execute auto purge. 実行中 "Running" screen appears.

1A-6

After auto purge is completed (check the remaining time is 0.0 min (27)), touch **閉じる** [Close] icon (28) to return the [Analysis Main Screen]. After that, lock the LCD screen again (See 0-8) and operate from the Lab solution of HPLC control PC.



1A-7

Change the flow rate (29) to an appropriate value in [Data Collection] tab and click **ポンプ ON** [Pump ON] icon to start the column equilibration. (e.g. Superdex200 increase 10/300 : 0.4 ~0.5 ml/min).



※When the column is equilibrated with other buffer, perform step 1A-1~7 with MilliQ [A line] and equilibrate the column with MilliQ for 5 minute. And then perform step 1A-1~7 with new buffer [A line]. When you want to change buffer from ligand-free buffer to new ligand-plus buffer, you could perform step 1A-1~7 with new ligand-plus buffer immediately.

1B Using your SEC column.

At the beginning of your BT, the BL staff set up the system as described below.

- HPLC system, UV spectrophotometer and PILATUS have been connected and started.
- HPLC system is equilibrated with MilliQ and the flow rate is set to low [for example 0.05 ml/min].
- Lab Solution (HPLC control software) can be operated by remote desktop on BL control PC

CAUTION: The equilibration of column at Lab. is recommended to save time.

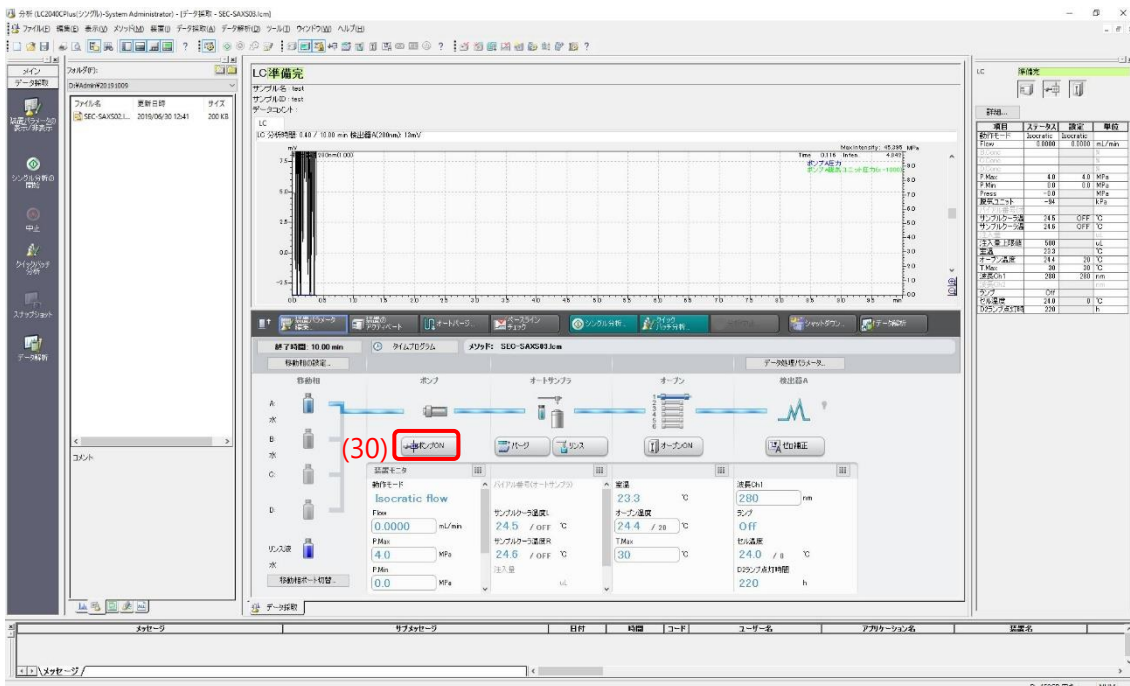
※When the column is stored with 20% ethanol etc.

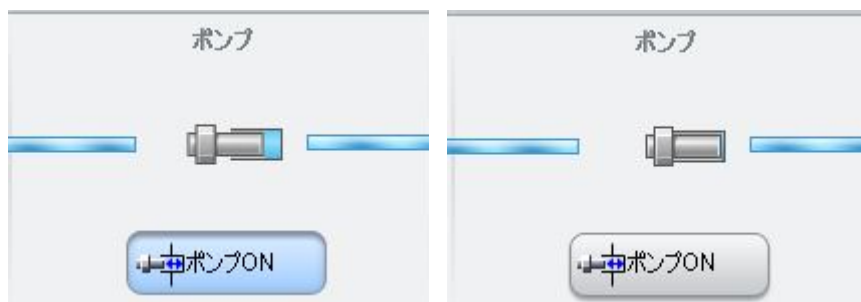
Perform step 1B-1~13with MilliQ [A line] and then wash column with more than 1 column volume MilliQ to eliminate ethanol in the column. Perform step 1B-1~7 with buffer [A line] and start the equilibration of column.

WARNING: Use filtered and degassed buffer: (<0.2 um filter). To degas buffer @Biophysics & Molecular physiology Lab. of PF again is recommended.

1B-1

Click **ポンプ ON [Pump ON]** icon (30) in **データ採取タブ [Data Collection]** tab at the lower middle of the Lab Solution screen to stop the pump. [Operating: light blue, stopped: gray]





1B-2

Wait until the pressure (Press) status (31) on the right side of the screen reaches 0M Pa.

項目	ステータス	設定	単位
動作モード	Isocratic	Isocratic	
Flow	0.0000	0.0000	mL/min
B.Conc			%
C.Conc			%
D.Conc			%
P.Max	4.0	4.0	MPa
P.Min	0.0	0.0	MPa
Press	-0.0	(31)	MPa
脱気ユニット	-94		kPa
ハイアル番号(オ)			
サンプルクーラ温	24.5	OFF	°C
サンプルクーラ温	24.6	OFF	°C
注入量			uL
注入量上限値	500		uL
室温	23.3		°C
オープン温度	24.4	20	°C
T.Max	30	30	°C
波長Ch1	280	280	nm
波長Ch2			nm
ランプ	Off		
セル温度	24.0	0	°C
D2ランプ点灯時	220		h

1B-3

Place your buffer bottle on the tray of the HPLC system. Put Solvent A line (cyan labelled) into your buffer bottle, and cover the spout of the bottle with parafilm.

1B-4

Unlock HPLC control from the front LCD panel (See 0-1), and touch パージ [Purge] icon (32) on 分析メイン画面 [Analysis Main Screen] to open オートパージ [Auto Purge] screen.

Turn on [1st] and リンス液 [Rinse] (light blue color indicates 'ON').

Enter purge time ([1st]: A, 3min, リンス液 [Rinse]: 15min).



1B-5

Touch **パージ開始** [Start Purge] icon (33) to execute auto purge. 実行中 "Running" screen appears.

1B-6

After auto purge is completed (check the remaining time is 0.0 min (34)), touch **閉じる** [Close] icon (35) to return the [Analysis Main Screen]. After that, lock the LCD screen again (See 0-8) and operate from the Lab solution of HPLC control PC.



1B-7

Change the flow rate (36) to 0.5 ml/min in [Data Collection] tab and click **ポンプ ON** [Pump ON] icon to start exchange HPLC system flow path (about 2 -3 min). (In the case of column equilibration, flow more than 1 C.V.)



1B-8

After about 2 - 3 min, set the flow rate to about 0.2 ml/min.

1B-9

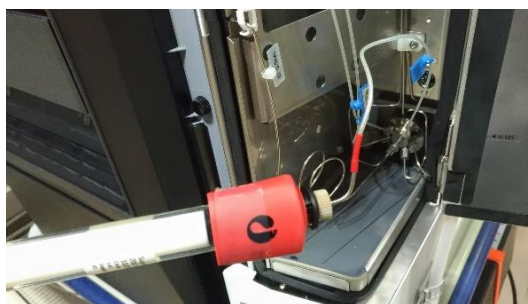
Open the column oven on the right side of the HPLC system and disconnect the union of line 1.
(The connector is 1/16 inches connector (No.10-32UNF, Tricorn column (GE) could connect directly.)

1B-10

Fill the column inlet with buffer using HPLC metal tubing (lower side tubing: upstream).

1B-11

Connect the column inlet to HPLC metal tubing. Tighten the ferrule while pressing on tubing, and after tightened, pull the tubing to make sure tubing doesn't disconnect.



1B-12

Connect the column outlet to HPLC plastic tubing (See 1B-10, 11)

1B-13

Place the column in the column oven. Install it upside down with masking tape.



1B-14

Wipe down the inlet and outlet with lab wipers and check for any leaks.

When closing the column oven door, set the tubing inside to prevent it from damage.



After the connection of column, set the flow rate and start the column equilibration.

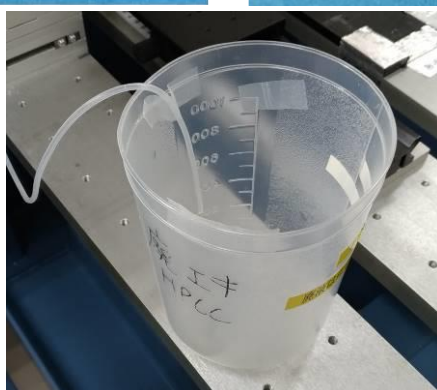
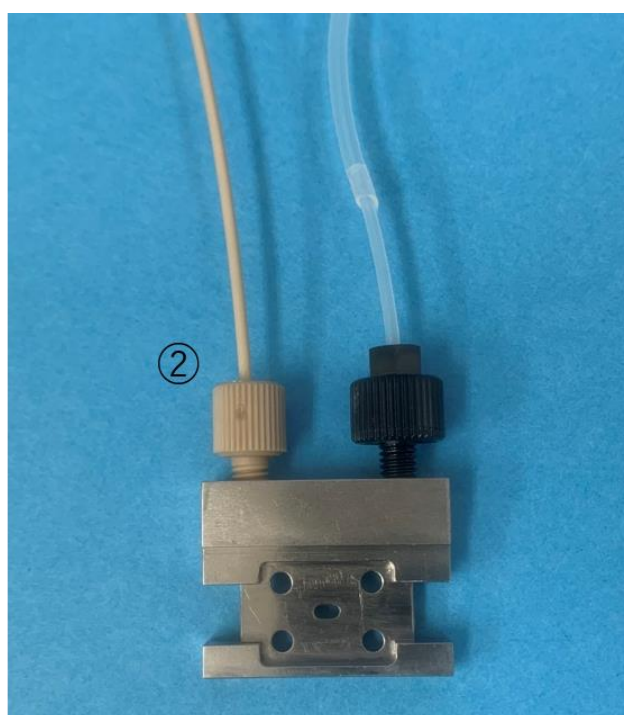
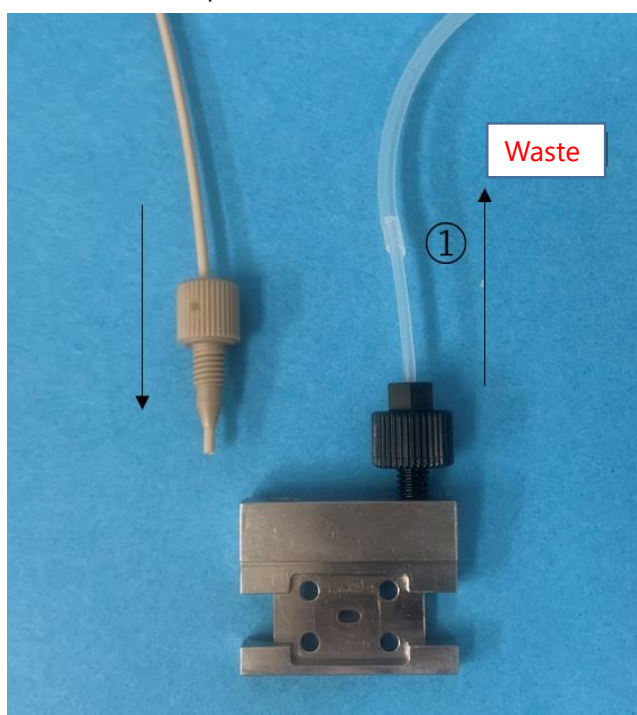
2 Preparation of SAXS flow cell and sample vials

2-1

If you want to perform the absolute scattering intensity calibration with water, you need to perform measurements of dark, air, empty cell and MilliQ, and then wash and dry SAXS flow cell with peristaltic pump and air pump.

2-2

The SAXS cell is equipped to both female fitting. **Connect the outlet of SAXS flow cell (black ferrule) to silicone tube first (1).** And set the waste cup on experimental bench. Fix the waste cup and silicone tube with adhesive tape.



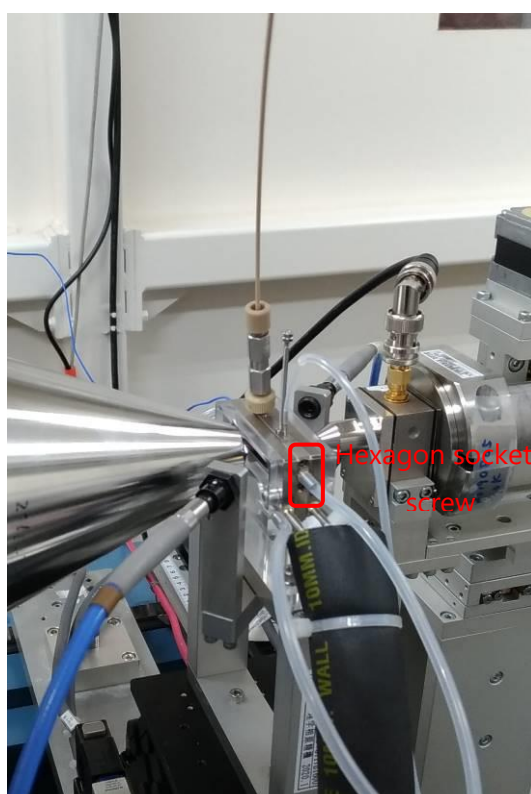
2-3

Connect the inlet of SAXS flow cell to PEEK ferrule (cream color) of HPLC (2).

(Note) Do not reverse the order of 2-2 and 2-3 to avoid the break of cell window.

2-4

Check no air bubbles in the flow cell window and flow path. Set flow cell in the cell holder properly and tighten 2 hexagon socket setscrews for flow cell fixation. To avoid tilting the cell position, fix the PEEK tube with the adhesive tape. Be sure not to apply excessive tension to the PEEK tubing.



2-5

Dispensing sample solution into sample vial. Samples should be filtered or supernatant after centrifugation (15krpm 15min). Dispense sample (the minimum volume is the injection volume + 10 ul) into the exclusive glass vial (water maximum recovery (P/N: 186000385c)).

(The maximum injection volume is 500 ul.)

Avoid making air bubbles in the bottom narrow section. (The yellow tip is useful for dispensing sample.)



2-6 Setting up sample plate

Draw out the rack A (left) of the auto sampler. Set the sample vial to No.1 position of the plate 1 (front plate).

Note: Do not use the rack B.



3 SEC-SAXS measurement

To measure SEC-SAXS data, three equipment (HPLC, UV spectrophotometer, PILATUS) are needed.

At current stage, after the injection start of HPLC, click run icon of PILATUS. UV spectrophotometer will start automatically.

PILATUS is controlled from BL measurement PC. HPLC and UV spectrophotometer are controlled with the remote desktop outside experimental hatch [HPLC: BL control PC, UV spectrophotometer: BL measurement PC.]

SAXS measurement of the background (buffer)

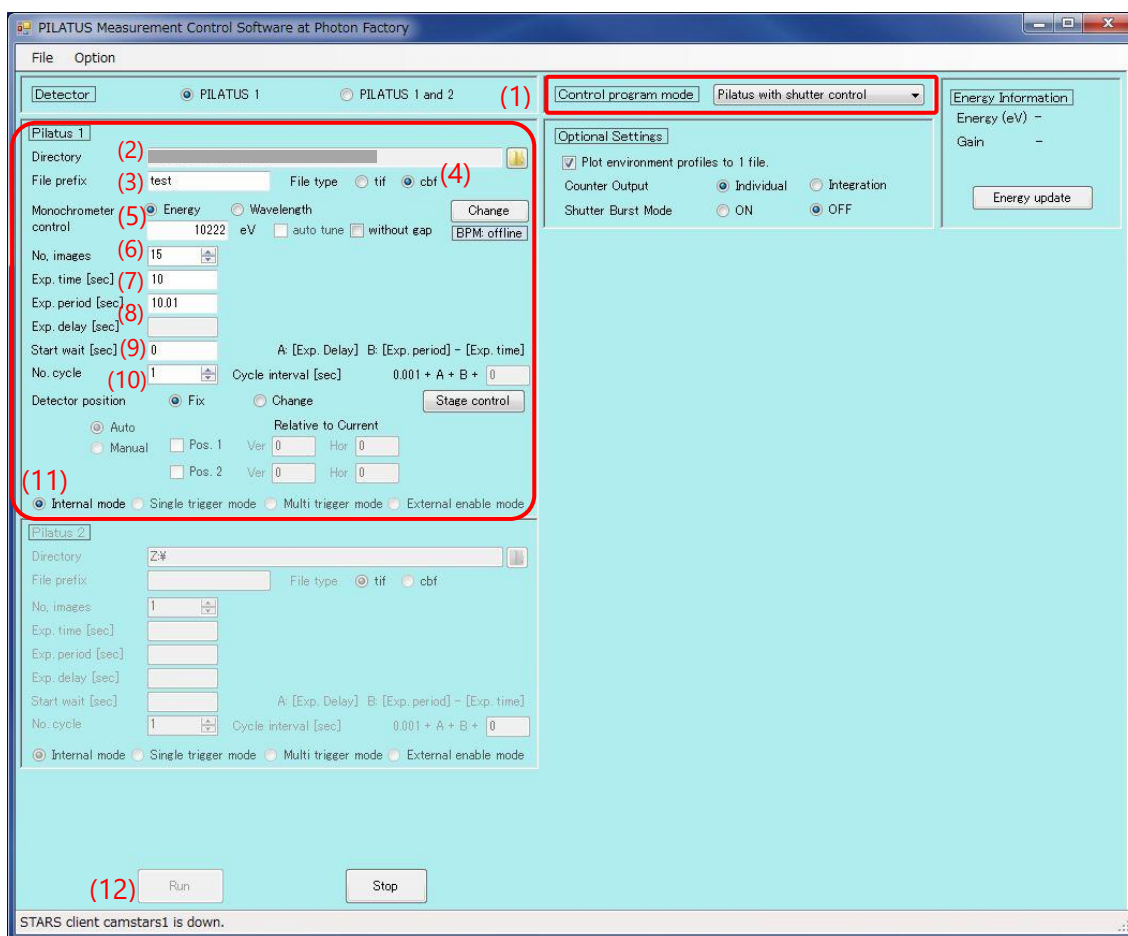
3-1 Lowering the flow rate

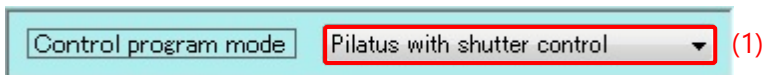
After the equilibration of the column, set the flow rate to **0.1 ml/min** @BL-15A2 ((same as measure at peak fraction.) **0.05 ml/min**@BL-10C) wait several minute until the pressure will be stable.

3-2

Confirmation [Control program mode] of PILATUS Measurement Control Software

Set [**PILATUS with shutter control**] in [Control program mode] (1) of PILATUS Measurement Control.





3-3

Measure SAXS image of background (buffer) [for example 10sec x 15 times@BL-15A2, 20sec x 15 times@BL-10C].

Set parameters in PILATUS Measurement Control Software as described below.

Directory [enter your folder] (2)

File Prefix [enter your file prefix] (3)

File type [select cbf or tif] (4) (If you want to process the data by other than SAngler, select tif.)

Monochromator control [select energy or wavelength, and enter appropriate value.] (5)

No. images [15] (6)

Exp. Time [10] @BL-15A2 default, [[20] @BL-10C] (7)

Exp. period [10.01] @BL-15A2 default, [[20.01] @BL-10C] (8)

Exp. delay : (This parameter is unable to be input with [PILATUS with shutter control] mode.)

Start wait 0 (For SEC-SAXS experiment) (9)

No. cycle 1 (For SEC-SAXS experiment) (10)

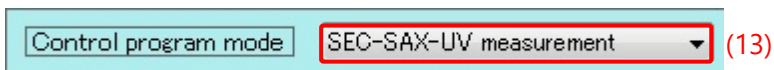
Check [internal mode] (11).

Close the experimental hatch to open X-ray shutter before start.

Click [Run] (12) to start measurement.

3-4

After back ground measurement, Set [SEC-SAXS-UV measurement] (13) in [Control program mode] of PILATUS Measurement Control to synchronize the start of PILATUS and UV spectrophotometer.



Setting of UV spectrophotometer software.

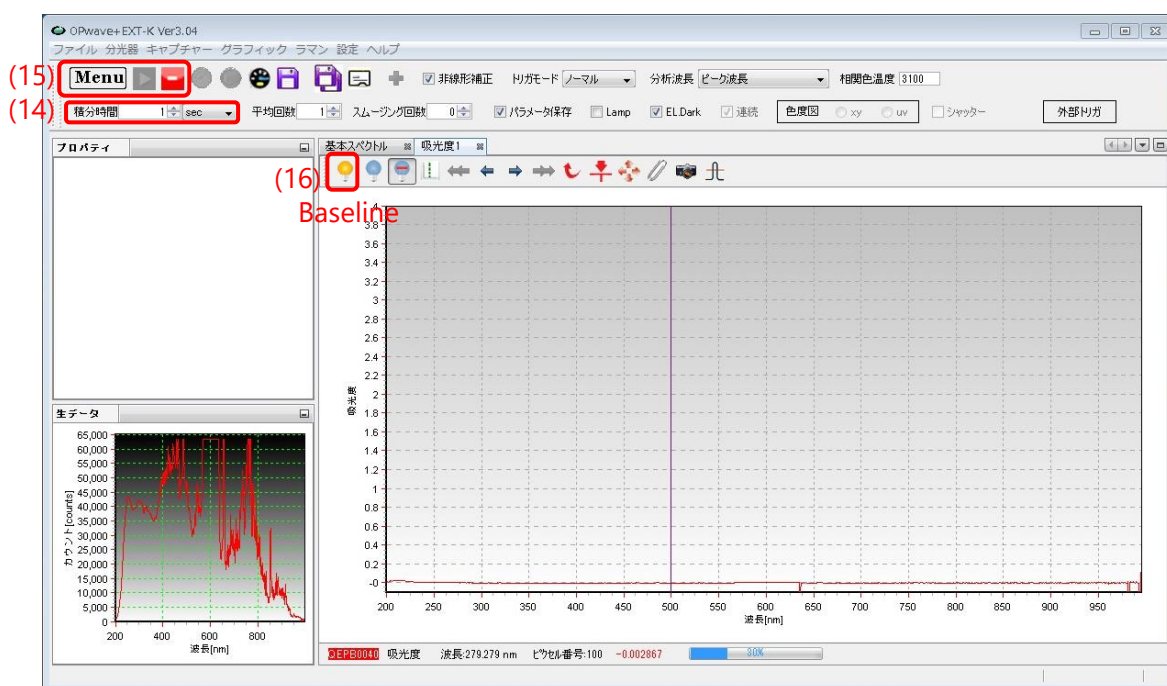
3-5 Initial setting of UV spectrophotometer software

Confirm that 積分時間 [integration period] is [1sec] (14) in OPwave+EXT-K (UV spectrophotometer software). If different period is set, the re-adjustment of the UV spectrophotometer is needed, see p.5 of OPwave manual or Appendix2.

Confirm that OPwave+EXT-K is [live mode].

If the icon next to menu icon is grey and the spectra is renewed every sec, OPwave+EXT-K is set to [live mode].

If the icon next to menu icon is green, Click that green icon (15), and set OPwave+EXT-K to [live mode].



3-6 Measurement of reference spectra with UV spectrophotometer

Check SAXS flow cell position and no air bubble in the flow path (See 2-4). And check the flow rate is 0.1 ml/min@BI-15A2 [0.05 ml/min@BL-10C]

And then click the yellow bulb icon (16) to get reference spectra.



Measurement Example

Column : Superdex200 increased 10/300 (CV: 24ml)

Flow rate 0.5 ml/min @0~14 ml (0~28 min) and 0.1 ml/min @14~16.5 ml (28~53min) @BL-15A2
(0.05 ml/min @14~16.5 ml (28~78min) @BL-10C).

(Measure peak fractions with lower flow rate.)

SAXS (expose 10sec/image, exp period 10.01sec)、

UV measurement period is set to 5sec as default (twice frequency as X-ray) @BL-15A2.

(exposure 20sec/image, exp period 20.01sec, UV 10sec@BL-10C)

Total number of measurements are

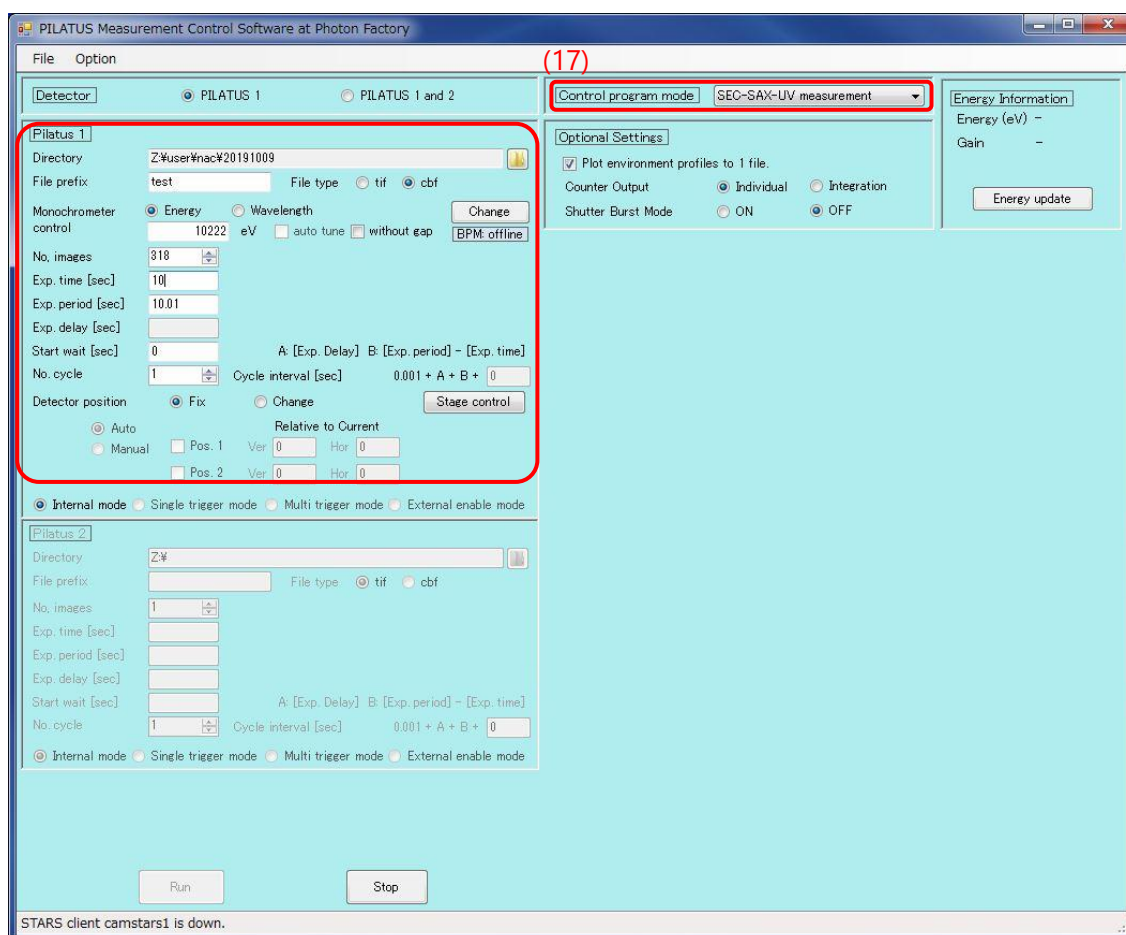
(SAXS) $53 \times 60 / 10.01 = 318$ @BL-15A2 [$78 \times 60 / 20.01 = 234$ @BL-10C]

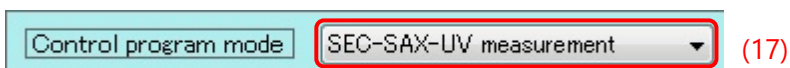
(UV) $53 \times 60 / 5 + 1 = 637$ @BL-15A2 [$78 \times 60 / 10 + 1 = 469$ @BL-10C]

Setting parameters of PILATUS measurement control software

3-7 Confirmation [Control program mode] of PILATUS Measurement Control Software

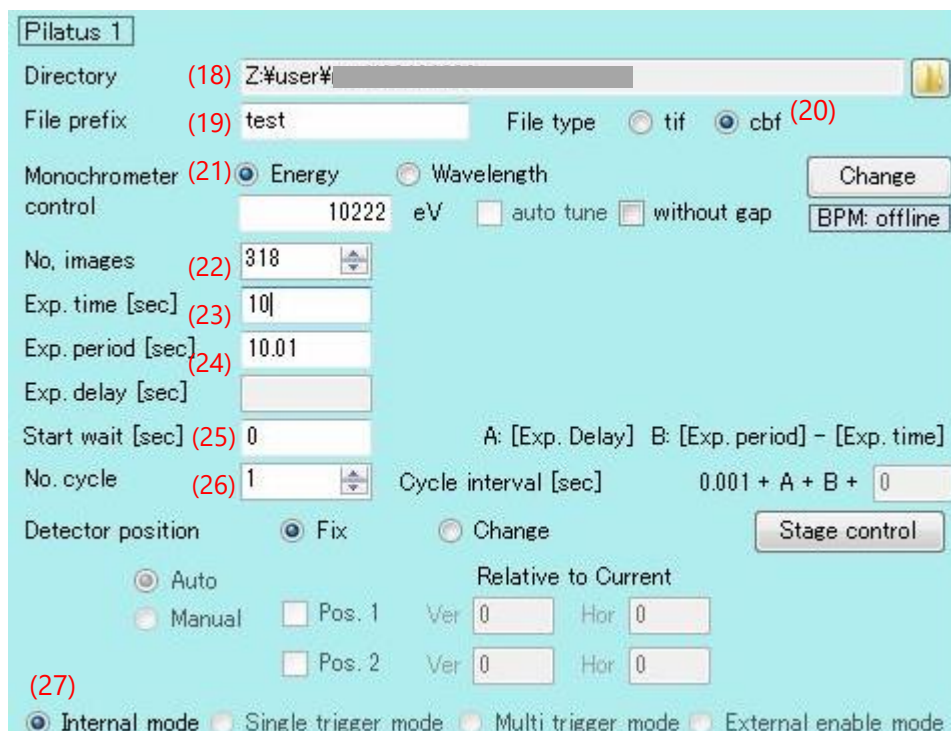
Set [SEC-SAXS-UV measurement] (17) in [Control program mode] of PILATUS Measurement Control.





3-8 Setting up of PILATUS measurement parameters

Set parameters in [PILATUS Measurement Control Software] as described below.



Directory [enter your folder] (18)

File Prefix [enter your file prefix] (19)

File type [select cbf or tif] (20) (If you want to process the data by other than SAngler, select tif.)

Monochromer control [select energy or wavelength, and enter appropriate value.] (21)

No. images [318] (22)

Exp. Time [10] @BL-15A2 default, [[20] @BL-10C] (23)

Exp. period [10.01] @BL-15A2 default, [[20.01] @BL-10C] (24)

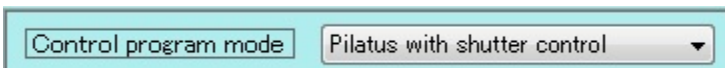
Exp. delay : (This parameter is unable to be input with [PILATUS with shutter control] mode.)

Start wait 0 (For SEC-SAXS experiment) (25)

No. cycle 1 (For SEC-SAXS experiment) (26)

Check [internal mode] (27).

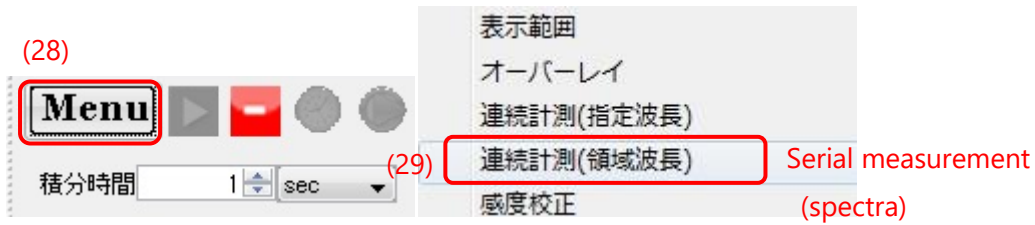
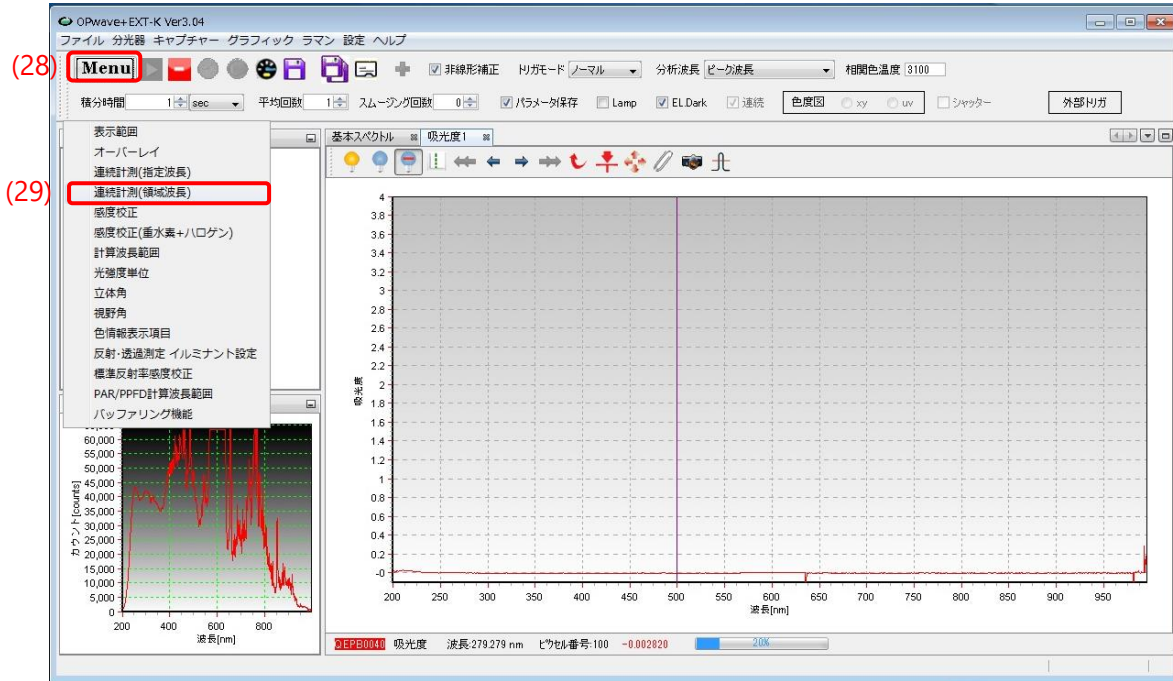
(Appendix) In this mode, you can start Spectroscopic and SAXS measurement simultaneously. If you want to start these two measurement independently, you will set "Control program mode" to "Pilatus with shutter control".



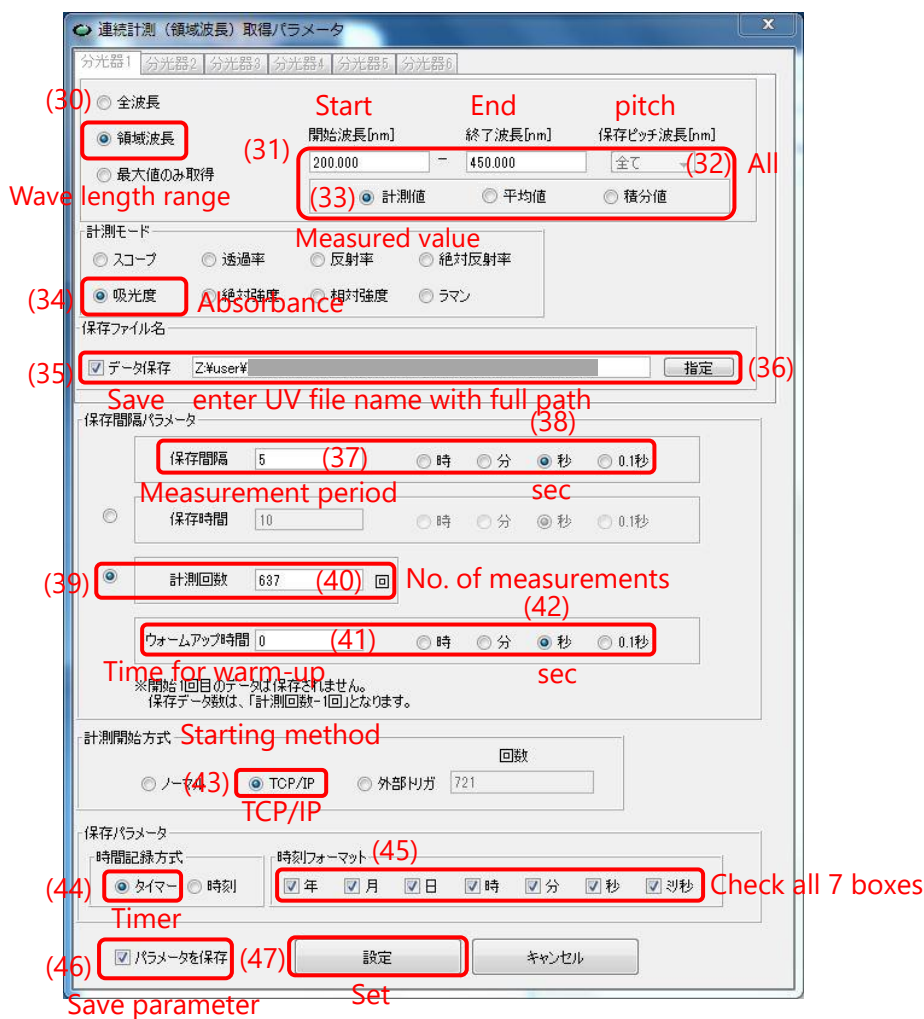
Setting parameters of OPwave+EXT-K

3-9 Setting parameters of OPwave+EXT-K

Click Menu icon (28), and select 連続計測(領域波長) [serial measurements (spectra)] (forth line) (29).
連続計測(領域波長) [serial measurements (spectra)] window will open.



Set parameters in the window as described below.



領域波長: [wave length range] (30) [200] – [450] nm (31)

保存ピッチ: [pitch] /全て: [all] (32), Check 計測値: [measured value] (33)

計測モード: [mode] /吸光度: [absorbance] (34)

保存ファイル名: [file name] / check データ保存: [check save data] (35), [enter UV file name (***_UV)] (36)

保存間隔パラメータ: [parameters for measurement period section]

保存間隔: [measurement period] / [5]@BL-15A2 sec [[10]sec@BL-10C] (37) and check 秒: [sec] (38).

Check 計測回数: [Check No. of measurements] (39), enter [[637] @BL-15A2] [[469]@BL-10C] (40)

ウォームアップ時間: [time for warm-up] / [0] (41) and check 秒: [sec] (42).

計測開始方式: [starting method] / Check [TCP/IP] (43).

保存パラメータ: [other parameters for saving section]

Check タイマー : Check [Timer] (44) and Check all 7 boxes in [時刻フォーマット]: time format (45)

Check パラメータを保存: [save parameters check box] (46).

Click 設定: [Set] icon (47).

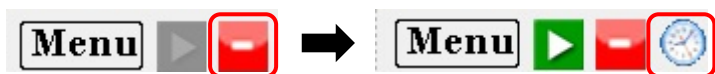
Notification window will open. Click OK.

3-10 preparation for serial measurement

Click [stop] icon (red icon) (49) to stop [live mode], and click [serial measurement] icon (clock icon) (50) to start waiting state for the start signal from PILUTAS.



(補足) If you want to start spectroscopic and SAXS measurement independently, you will set [計測開始方式] to “ノーマル”. When you click 「設定」 button, you can start measurement by clicking [serial measurement] icon (clock icon)(50) after stopping the live mode (red icon (49)).

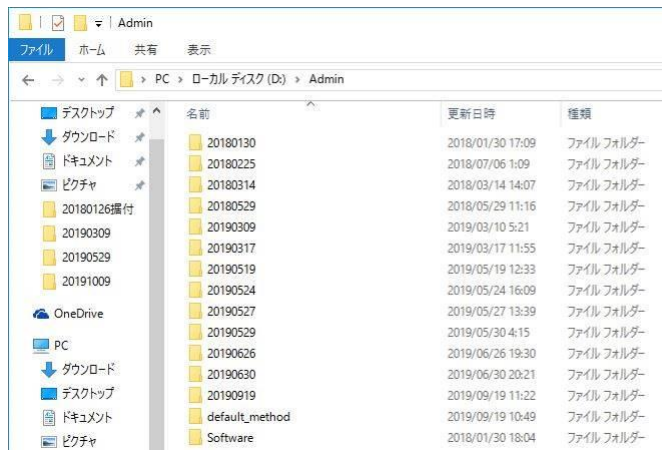


Setting of HPLC

3-11 (one time for each beam time)

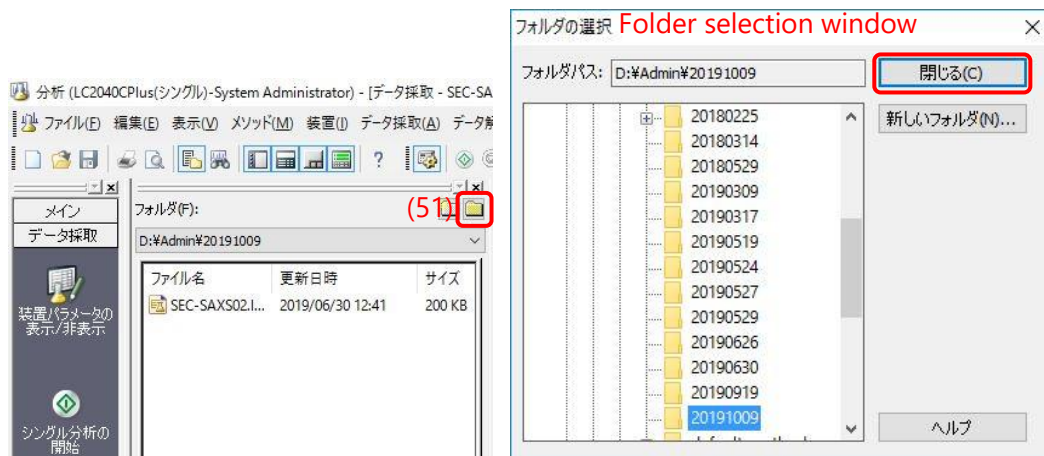
Create a new "YYYYMMDD" folder in [D:¥¥Admin¥] on Windows and

Copy [D: ¥¥Admin¥default_method¥SEC_SAXS02.lcm] to the new "YYYYMMDD" folder.



3-12 (one time for each beam time)

Click the folder icon (51) on the left side of Lab Solution to open the folder selection window and select the folder which you created in 3-11. Then, double-click SEC-SAXS02.lcm in that folder.



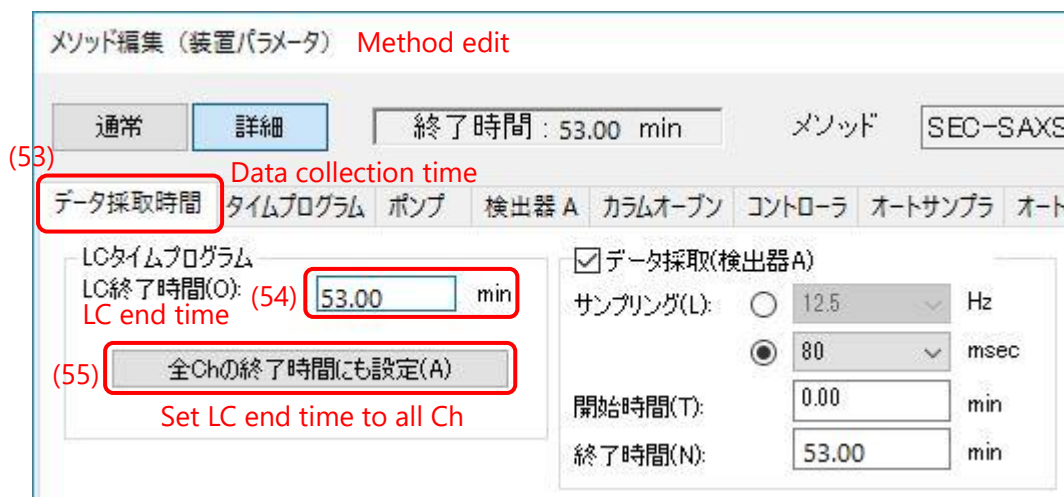
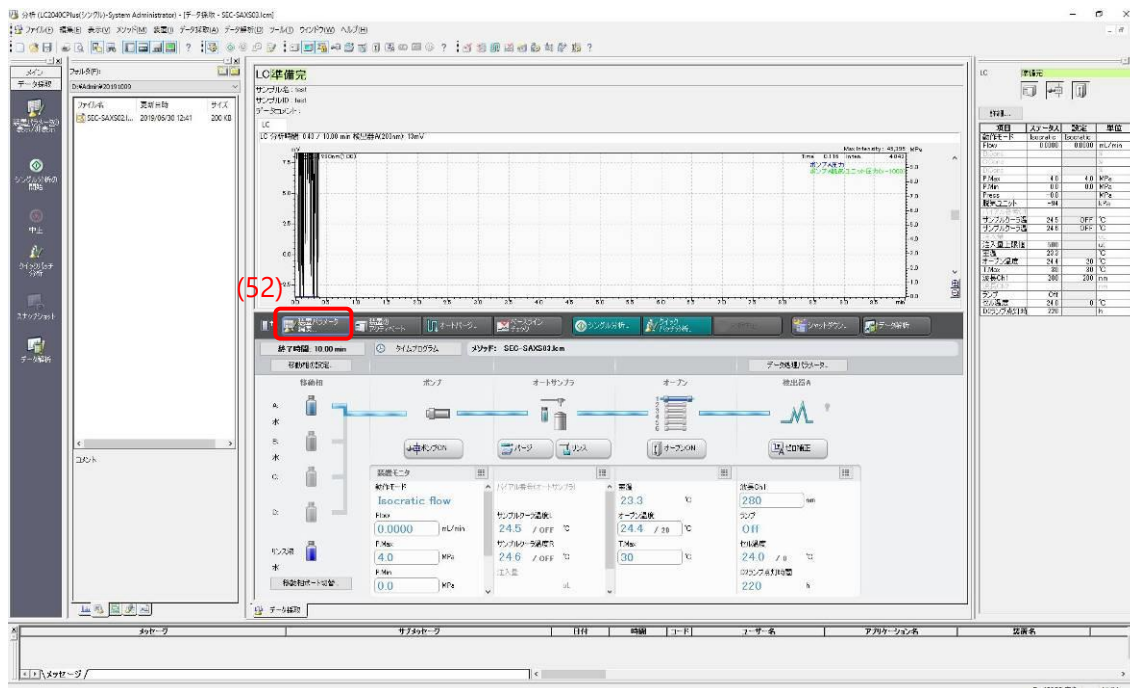
3-13

Click 装置パラメータ編集 [Instrument Parameter Edit] tab (52) in the center of the Lab Solution window to open メソッド編集 [Method edit] window.

Click データ採取時間 [Data Collection Time] tab(53) in メソッド編集 [Method edit] window.

Enter LC 終了時間 [LC end time]: 53@BL-15A2 min [78@BL-10C] (54).

And click 全 Ch の終了時間にも設定 [Set LC end time to all Ch] icon (55).



3-14

Click タイムプログラム [program] tab (56) in the method edit window, and set flow rate program.

	[時間]:Time (min)	ユニット:Unit	処理命令:command	数値:Value
1	27.99	ポンプ:Pump	Flow	0.5
2	28.00	ポンプ:Pump	Flow	0.1
3	53@BL-15A2	コントローラ:Controller	Stop	

メソッド編集 (装置パラメータ)

通常 詳細 終了時間 : 53.00 min メソッド SEC-SAXS02_test2.lcm 装置ヘダダウンロード

データ採 (56) タイムプログラム ポンプ 検出器 A カラムオープン コントローラ オートサンブラ オートバージ

Program

Max Intensity : 0 mL/min °C

時間	ユニット	処理命令	数値
1 27.99	ポンプ	Flow	0.5
2 28.00	ポンプ	Flow	0.1
3 53.00	コントローラ	Stop	
4 0.00			
5 0.00			
6 0.00			
7 0.00			
8 0.00			
9 0.00			
10 0.00			
11 0.00			
12 0.00			

クromatogram参照(L)...
グラジエントカーブ(D)

装置ヘダダウンロードして閉じる 閉じる ヘルプ

3-15

Click **ポンプ** [pump] tab (57) in the method edit window.

モード[Mode]: Isocratic flow

Flow: **0.5** ml/min [initial flow rate] (58)

ポート[Port]: A

移動相 [mobile phase]: 水[Water]

P.Max: **4.0** MPa (default: See the manual of the your SEC column) (59)

P.Min: **0.0** MPa (default: See the manual of the your SEC column) (60)

Method Edit (Device Parameters)

通常 詳細 終了時間: 53.00 min メソッド: SEC-SAXS02.lci

データ採取時間 タイムプログラム **ポンプ** (57) **検出器 A** (61) カラムオープン コントローラ オートサンブラ オートバージ

モード(M): Isocratic flow **Pump Detector A**

Flow(F): (58) 0.5000 mL/min

ポート

移動相

A 水

B 水

C

D

ポンプの設定

Pump: LC-2040 Pump

圧力の限界

P.Max(X): 4.0 MPa (59)

P.Min(N): 0.0 MPa (60)

圧縮率設定(S)...

3-16

Click 検出器 A [detector A] tab (61) in the method edit window.

セル部温調温度 [UV cell temperature]: Put a check mark and enter 30°C (62)

波長 [wavelength of Ch1]: 280 nm (63)

Method Edit (Equipment Parameters)

通常 詳細 終了時間: 53.00 min

データ採取時間 タイムプログラム ポンプ 検出器 A カラム

モデル: LC-2040 UV Detector

ランプ(L): D2

ポリティイ(P): +

レスポンス(R): 0.1 sec

(62) セル部温調温度(C): 30 °C UV cell temp

(63) 波長Ch1(1): 280 nm Wavelength of Ch1

アウトプット

強度単位(U): AU

AUXレンジ1(1): 1.0 AU/V

3-17

Click カラムオーブン [column oven] tab (64) in the method edit window.

Put a check mark on カラムオーブン [column oven] (65).

オーブン温度 [temp of column oven]: 20 °C [enter appropriate value] (66)

上限温度 [upper temp limit]: 30 °C (67) (when column oven temperature is set to 4~25°C)

Method Edit (Equipment Parameters)

通常 詳細 終了時間: 53.00 min メソッド: SEC-SAXS02.lcm

データ採取時間 タイムプログラム ポンプ 検出器 A カラムオーブン コントローラ オートサンブラ オートバージ

モデル: LC-2040 Oven Column oven Column oven

(65) カラムオーブン(V) (67)

(66) オーブン温度(O): 20 °C 上限温度(M): 30 °C

Temp of column oven Upper temp limit

バルブ(L): 6Position 1

3-18

Click コントローラ [controller] tab (68) in the method edit window.

Put a check mark on 脱気ユニット[degasser] (69) .



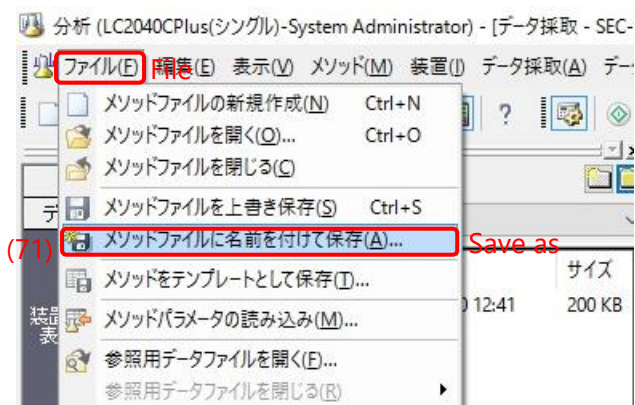
3-20

Click 装置へダウンロードして閉じる[Download to HPLC and close] (70) in the method edit window to send the edited method to HPLC.



3-21

In Lab Solution, select ファイル [File] ->メソッドファイルに名前をつけて保存 [Save as] (5th line) (71), then save edited method file as new file.



Execution of SEC-SAXS

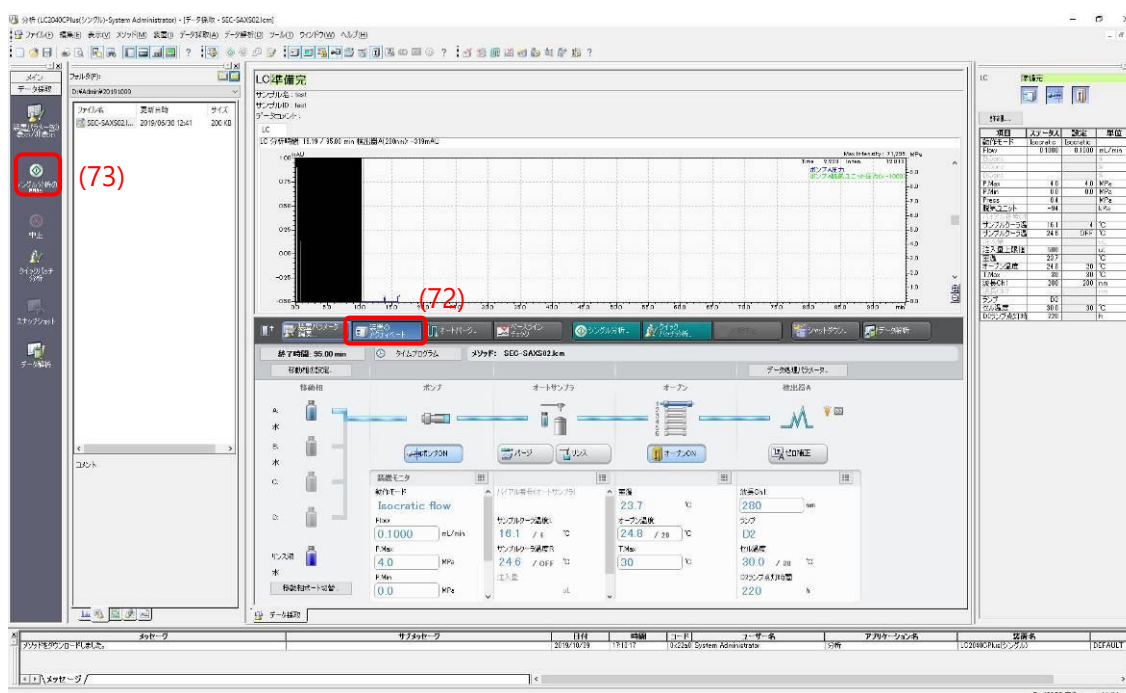
3-22 Start of the HPLC injection

Prepare SAXS flow cell and sample vial (See 2-1~6). Close the experimental hatch and open DSS.

3-23

Click 装置のアクティベート [activation icon] (72) in the center of the Lab Solution window to start flow which is set as initial condition of the edited method.

The activate icon changes to light blue color when HPLC starts.



3-24

Click シングル分析の開始 [single analysis] icon (73) in the left side column of Lab Solution window to open single analysis window. And enter parameters in the single analysis window.

サンプル名 [sample name]: enter sample name (74)

サンプル ID [sample ID]: enter sample id (sample id same as sample name is acceptable) (75)

メソッドファイル [method file]: confirm that method file is saved at 3-20 (76)

データファイル[Data file]: 保存先 [folder]: set folder location [D:¥Admin¥yyyyymmdd] (3-11,12) (77)
Enter file name [xxxxx.lcd] (78).

バイアル[No. of vial]: 1 [enter No. of vial, default 1] (79)

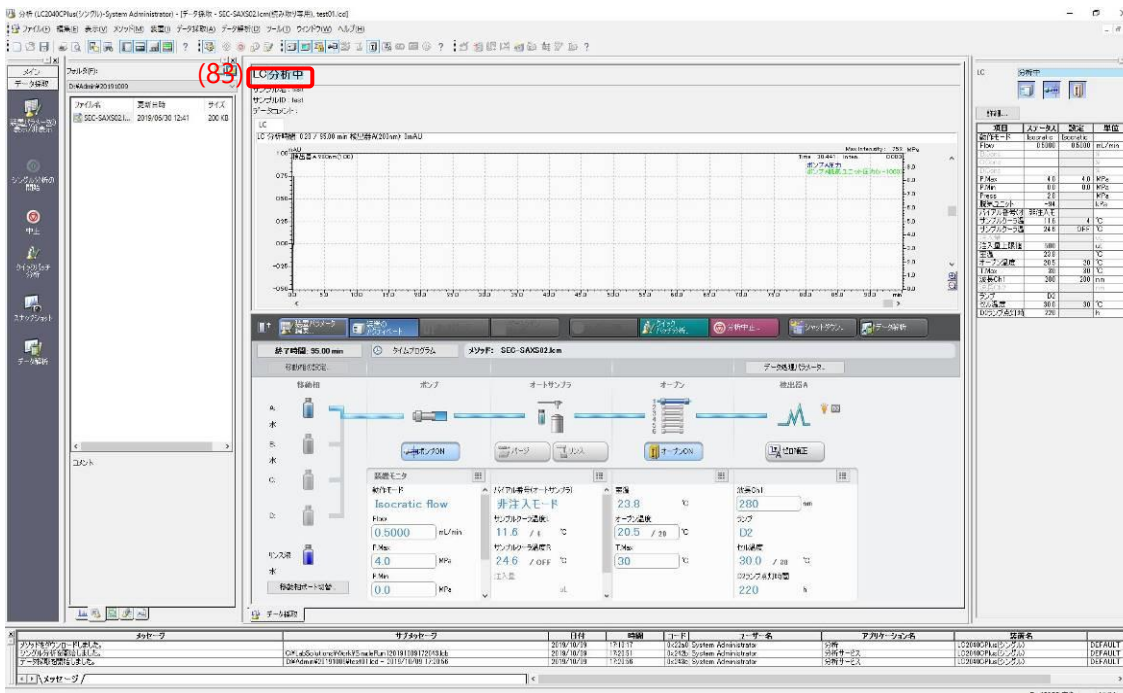
トレイ [No. of tray]: 1 [default 1] (80)

注入量 [injection volume]: 200 ul [enter injection volume, max 500 ul] (81)

3-25

Click OK bottom (82) in the single analysis window to start injection of HPLC.

When the HPLC injection is started, LC 分析中[LC analysis in progress] is displayed on the Lab Solution window (83).



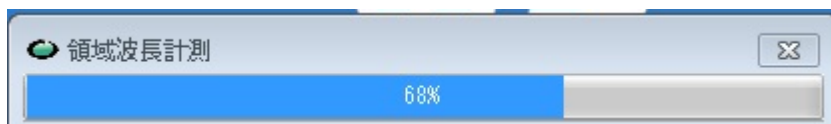
LC分析中 LC analysis in progress

3-24 Starting PILATUS measurement

Immediately after starting UPLC, click [Run] in PILATUS measurement software to start PILATUS. While PILATUS is running, a part of PILATUS measurement soft window will become pink in color and measured images will be displayed on the PILATUS image viewer software window. (You need to change PILATUS image viewer software to [measurement mode].)

3-25

Confirm the serial measurement of UV spectrophotometer start simultaneously, while the serial measurement of UV spectrophotometer, the progress bar window will open and update spectra on OPwave+EXT-K window.



3-26 Preparation for next measurement

After the SEC-SAXS measurement finish, re-set appropriate flow rate to re-equilibrate the column on Lab solution (because HPLC continue the flow at initial programmed condition.)

3-27

After the SEC-SAXS measurement finish, Notification window will open on OPwave+EXT-K, click OK and re-start [live mode] again by clicking [green start icon] (84) of OPwave+EXT-K.



3-28

Return to 1 to start the equilibration with another buffer or return to 2-4 to repeat experiments with same buffer.

4 Cleaning

4-1

Exchange A line to MilliQ [See 1A-1~7].

And then flow MilliQ [A line] at low flow rate (0.05ml/min with Superdex 200 increase 10/300).

*Keep eye on the remaining amount of MilliQ.

4-2 Disposal of waste fluid (waste cup and brown glass bottle), pipette tips and vials.

- Waste fluid: plastic container for hydrous organic solvents [in case of buffer without heavy metal ions] @ Chemistry Lab. of PF.
The primary and secondary washing solution must be collected in the plastic container. And then rinse cup and bottle with tap water and place them on the shelf @ Biophysics & Molecular physiology Lab. of PF.
- Pipette tips and vial caps: Trash can for cap, plastics @ Biophysics & Molecular physiology Lab. of PF.
- Vial: Trash can for glassware @ Biophysics & Molecular physiology Lab. of PF.

4-3

Wash the flow cell with peristaltic pump and dry it with air pump.

Rinse the unions and ferrules with MilliQ.

If you find a stain on the window of the flow cell, note it on the log book.

FAQ

1) The UV drift on OPwave-EXT-K is large.

The UV baseline is changed by flow rate. Therefore UV base line must be set at the same flow rate as the sample elution period.

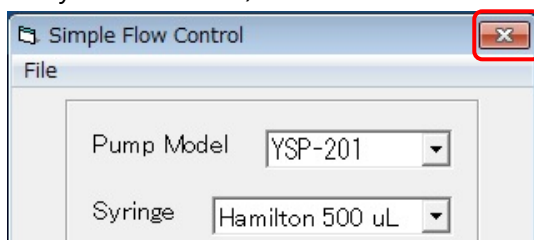
In case of unstable and large UV drift, try to change SAXS flow cell.

2) OPWave-EXT-K is frozen or stacked.

See Appendix2, and restart OPWave-EXT-K and perform initial setting.

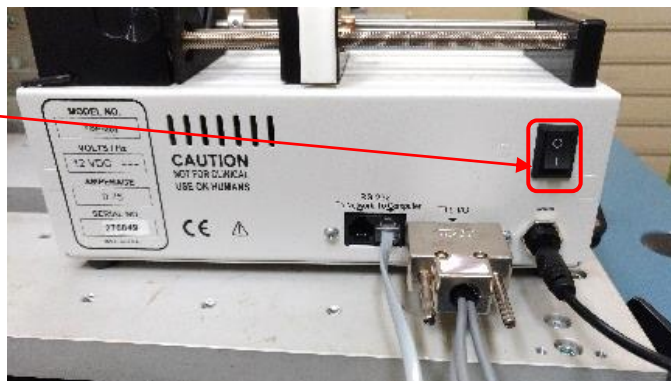
3) OPWave-EXT-K and PILATUS measurement software are not synchronized.

Close "Simple Flow Control" (Flow system software) on OPWave-EXT-K running Windows PC.



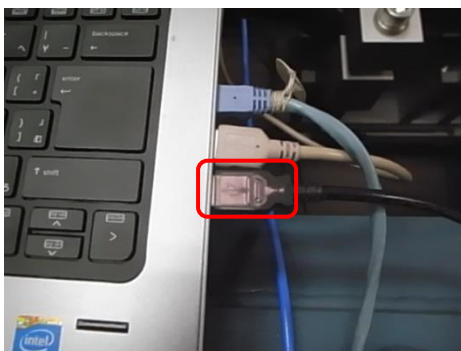
Turn off the syringe pump (backside of the syringe pump).

Switch



Disconnect USB cable (black cable) of the syringe pump from Windows note PC in the experimental hatch.

Do not disconnect USB cable of UV spectrophotometer.



Appendix 1 Solvent Inlet Line

HPLC system has five solvent inlet line.

Solvent A (cyan), B, C and D: buffer line, [B, C, D (not in use) -> 50% Ethanol]

RINSE(black) : rinse

Please set solvent inlet lines as described below.

CAUTION: Change the bottles for the inlet line after the flow was stopped.

(1) During storage

20% Ethanol : A(cyan), RINSE(black)

(2) During flow MilliQ

MilliQ Water: A(cyan), RINSE(black)

(3) During flow buffer

MilliQ Water: RINSE(black)

buffer: A(cyan)

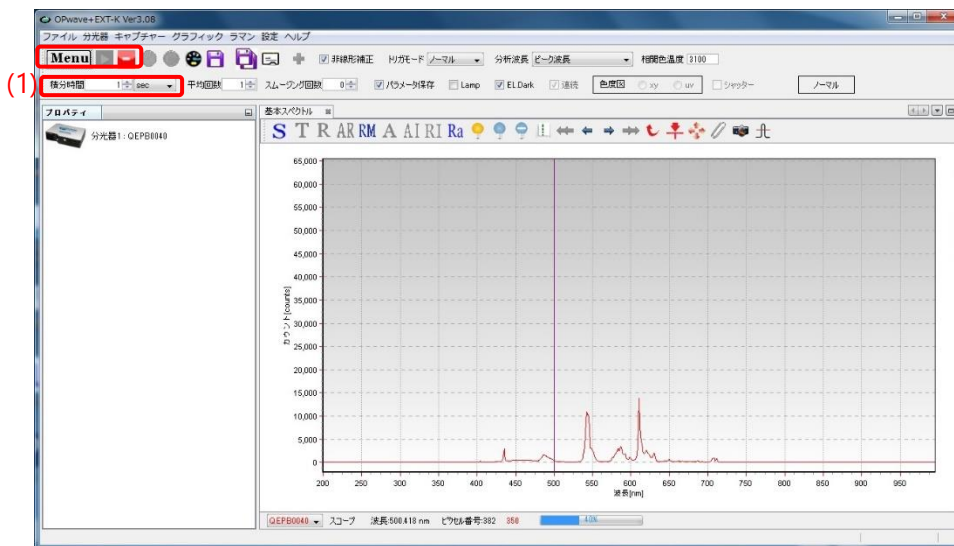
	A (cyan)	RINSE(black)	B, C, D
Storage	20% Ethanol	20% Ethanol	50% Ethanol
MilliQ	MilliQ	MilliQ	50% Ethanol
buffer	buffer	MilliQ	50% Ethanol

Appendix 2 How to start UV spectrophotometer control software [OPwave+EXT-K]

2.1. Click OPwave+EXT-K icon.



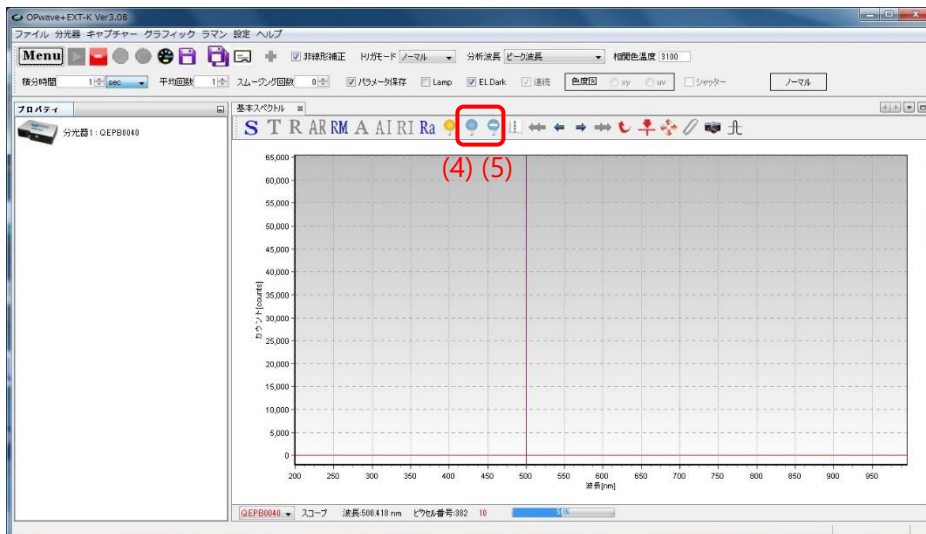
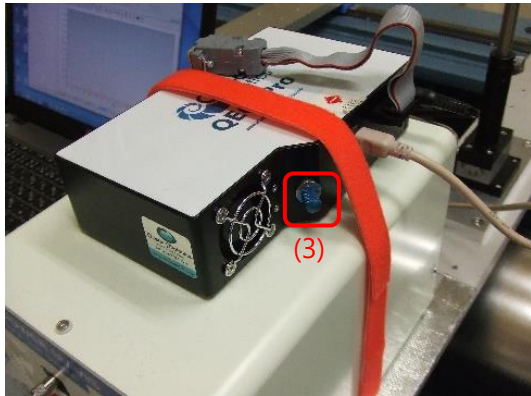
2.2. Confirm that 積分時間: [integration period] is [1sec] in OPwave+EXT-K (UV spectrophotometer software). If not, set [1sec] (1).



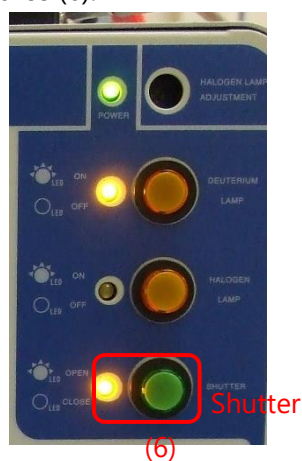
2.3. Confirm that OPwave+EXT-K is [live mode]. If the icon next to menu icon is grey and the spectra is renewed every sec, OPwave+EXT-K is set to [live mode]. If the icon next to menu icon is green, Click that green icon (2), and set OPwave+EXT-K to [live mode].



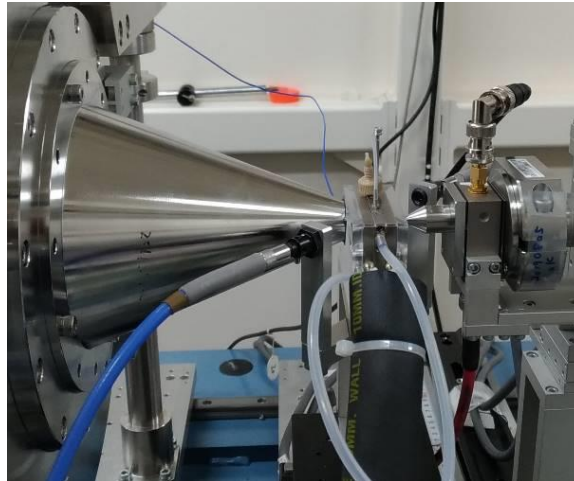
2.4. Loosen and remove light fiber cable from UV spectrophotometer, and install metal cap to shield from light (3). Click dark save icon (blue bulb) and click minus dark icon (blue bulb with white bar). The icon will be changed to blue bulb with red bar.



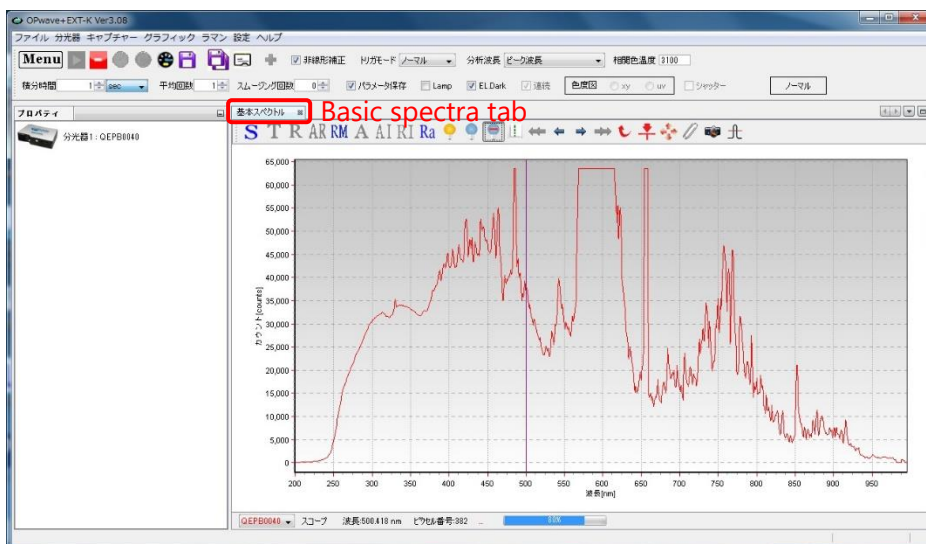
2.5. Re-connect UV spectrophotometer and light fiber cable. Confirm that the shutter of light source is open, if not, push shutter button of light source (6).



2.6. Set SAXS flow cell (filled up with MilliQ or buffer) to cell holder.



2.7. Select 基本スペクトル: [Basic spectra] tab in OPwave+EXT-K.



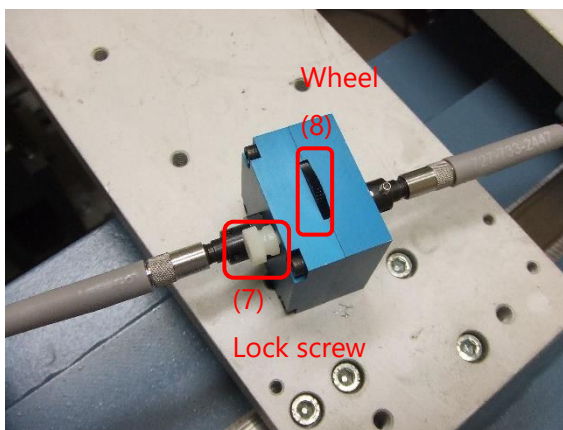
2.8. Adjust light intensity with attenuator (actual maximum counts should be less than 70~80 % of maximum (QEpro@BL-15A2: 18bit, QE65Pro@BL-10C: 16bit). [This step can be skipped. Because crash of Op-wave doesn't affect the light intensity.]

(Example: when transparent protein solution is measured (range 220 – 400 nm), adjust about 120000 counts (QEpro) and 30000 counts (QE65Pro) [about 70% of maximum]).

How to adjust with attenuator

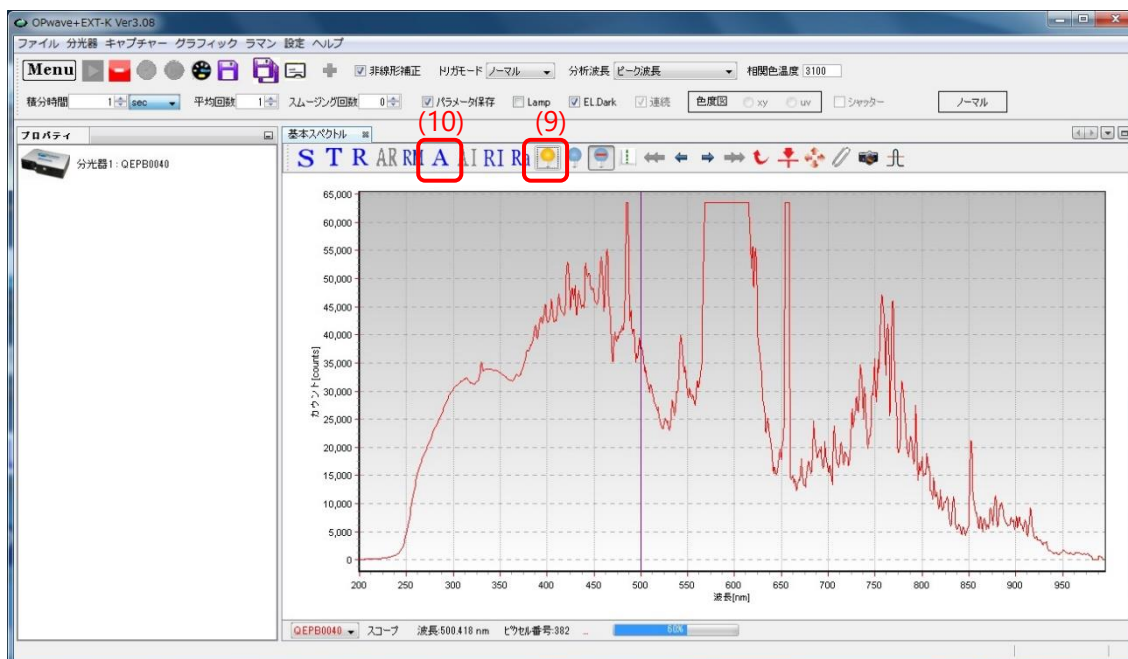
Loosen white lock screw (7) and rotate black wheel (8) to adjust aperture.

(When the lock screw will be tightened, light intensity is easy to change.)



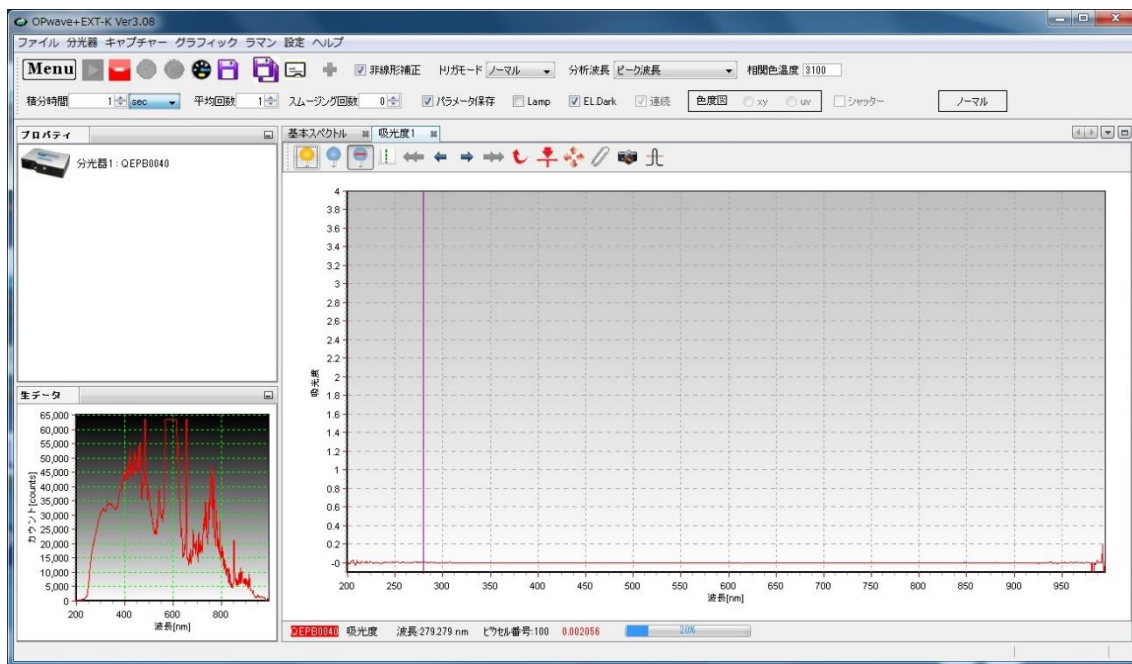
2.9. Click baseline icon (yellow bulb) (9) to make reference, and then click [A] icon (Absorbance mode) (10) to make absorbance tab. select 吸光度 1: [absorbance 1] tab (11).

The reference at the slow flow rate on SEC-SAXS experiment is needed for UV spectra measurement, but this step is adequate operation check at startup.



baseline A(Absorbance mode)





2.10. Click the remote desktop icon of the measurement PC outside experimental hatch.

- UV for HPLC & Solution Flow@BL-15A2
- Flow&QE65pro Remote Desktop@BL-10C

