ULSAN NATIONAL INSTITUTE OF SCIENCE AND TECHNOLOGY

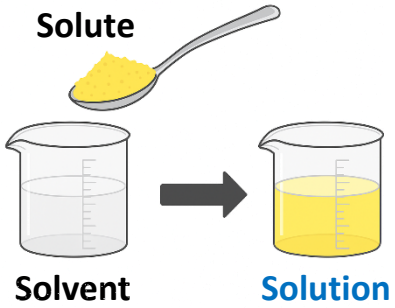
Nuclear Magnetic Resonance spectroscopy

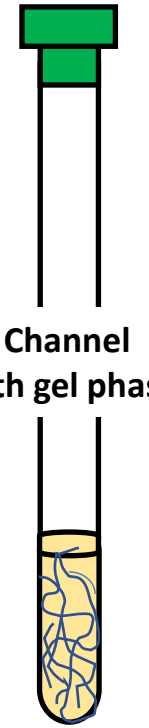
Normal Operation Training Course

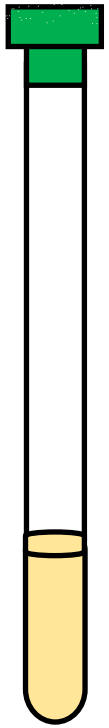
Pre-Preatment

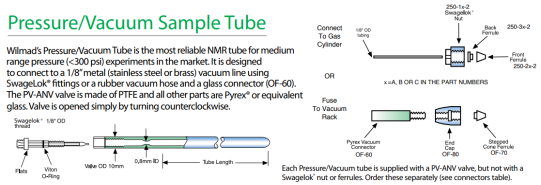
Sampling

Solution NMR



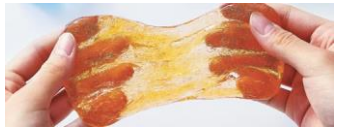




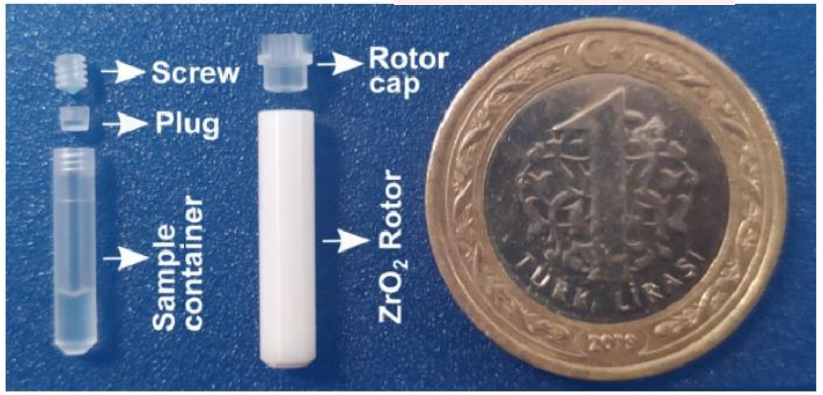


Pressure tube

HR-MAS NMR

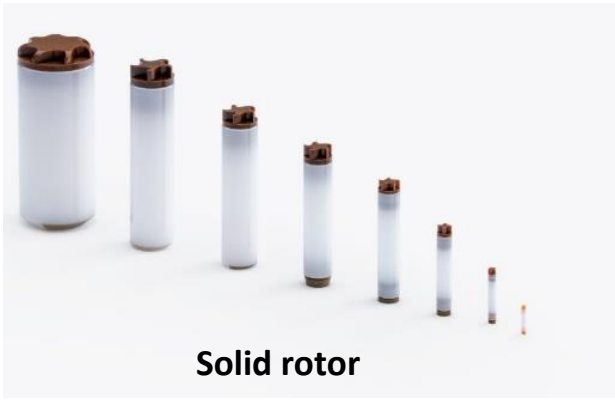


Gel & Paste



HR-MAS insert kit

Max. MAS Speed [kHz]	Rotor Diameter [mm]	Rotor Volume [µl] (Rounded)
7	7	360
15	4	106
24	3.2	47
42	1.9	13
67	1.3	3
111	0.7	0.6
160	0.4	0.12



Solid rotor

Solid-state NMR

Solid
(Powder, film, fiber)

Fine powder

Flexible film

Sampling (Solution NMR)

1) Normal case

Sample(~ 20mg) + Deuterated solvent (500uL)
Deuterated solvent: ex) D2O, CDCl3, DMSO-D6

2) 10% D solvent (Quantitative NMR with Calibration curve)

Sample(~ 20mg) + Non-D solvent (450uL) + Deuterated solvent (50uL)
Non-D solvent: H2O, CHCl3, DMSO-H6

3) After reaction in water or solvent base without pretreatment

Sample(~ 20mg) + Non-D solvent(500uL)
Non-D solvent: H2O, CHCl3, DMSO-H6

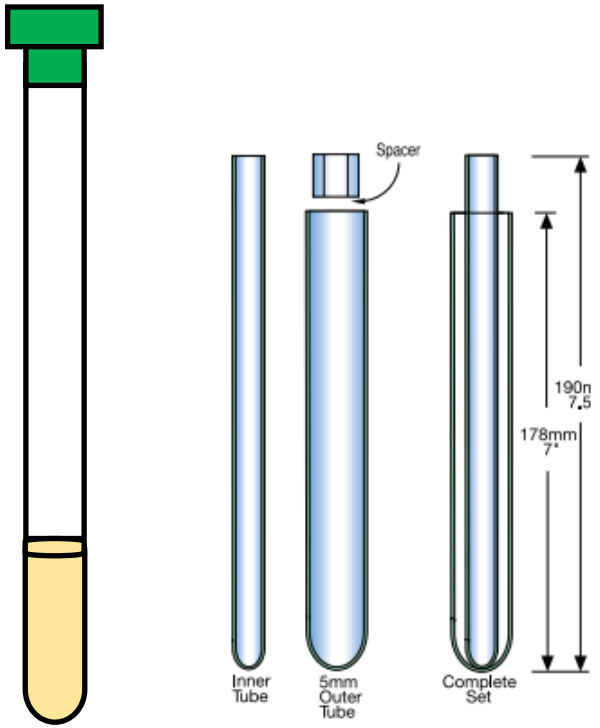
4) Coaxial tube

Inner tube: NMR solvent or Reference (Chemical shift, Concentration)
Outer tube: Sample (Non-D solvent, unknown concentration)

5) Gelation with coaxial tube / without coxial tube

With coxial tube
Inner tube: Deuterated solvent
Outer tube: Sample

6) Rolling flim



NMR sample tube Coxial tube

Sampling (Solid-state NMR)

Figure 1: MAS rotors with different diameters for different spinning speeds

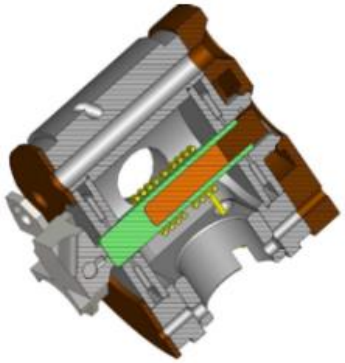


Figure 2: Schematic of a Bruker MAS stator

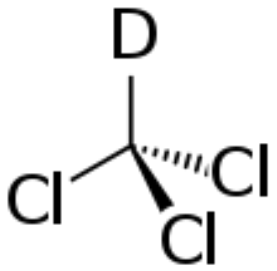
Max. MAS Speed [kHz]	Rotor Diameter [mm]	Rotor Volume [µl] (Rounded)
7	7	360
15	4	106
24	3.2	47
42	1.9	13
67	1.3	3
111	0.7	0.6
160	0.4	0.12

https://www.bruker.com/en/products-and-solutions/mr/nmr/probes/Solids-Probes.html

NMR solvent

Dielectric constant : Polar > 20 > Non-polar

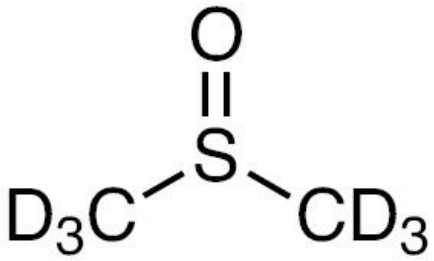
NMR Solvent Storage
Avoid light & moisture: All NMR solvent
Store refrigerator (4°C): CDCl3, THF-D8



CDCl3
- Dielectric constant: 4.8

- Advantage:
  - Cheap,
  - Easily Removed

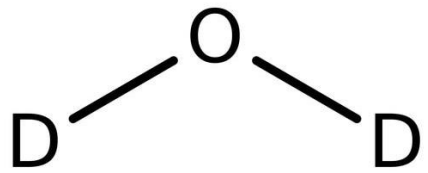
- Disadvantage:
  - Light sensitive(Contain HCl)
  - Toxic, carcinogenic
  - Weak lock signal
  - Easily evaporated



DMSO-D6
- Dielectric constant: 46.7

- Advantage:
  - Strong lock signal

- Disadvantage:
  - High price
  - Highly viscosity
  - Easily contain water
  - M.P.: 20 ~ 22 °C (Freeze RT)
  - Hard removed



D2O
- Dielectric constant: 78.5
- Advantage:
  - Cheap

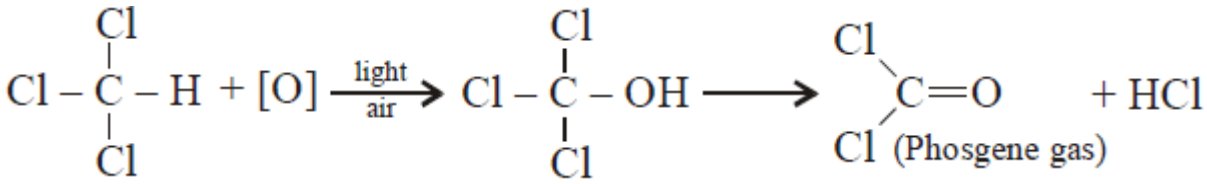
- Disadvantage:
  - Exchange OH and NH
  - Easily contain water
  - Chemical shift (Temp. dependent)
  - Hard removed

https://m.blog.naver.com/PostView.naver?isHttpsRedirect=true&blogId=release&logNo=50091986445

Caution

CDCl3 + Light -> HCl

- Use silver foil
  (Stabilizer, radical scavenger)
- Use molecular sieve
  (Eliminate H2O)



Change of chemical shift

- Temperature
- pH
- Concentration

HOD peaks

- Related residual H2O
- Slow exchange H2O and HOD
  → two peaks
  (singlet H2O, 1:1:1 triplet HOD)
- Hydrogen bond
  → Broad peak of HOD

How to remove residual water

→ Use Molecular Sieve 3~5 g in 100g (CDCl3)

- 3 Å: Adsorption of polar liquid (NH3, H2O)
- 4 Å: Adsorption of Non-polar liquid
  (H2O, CO2, SO2, H2S, C2H4, C2H6, C3H6, EtOH)
- 5 Å: Adsorption of Linear CxHx to n-C4H10
  Alcohols to C4H9OH
  C4H9SH

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Quality of sample tube

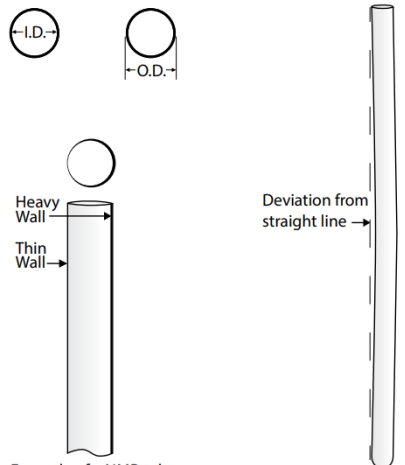
Thin wall (Precision-Glass)
- **Temp 120 °C**
- Critical shimming quality (low Fe2O3)
- **Good volume reproducibility**
- High sample volume in RF coil

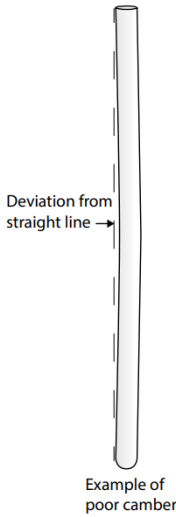
• 535-PP-7 (600 MHz grade, $40)
• 527-PP-7 (400 MHz grade, $25)

Economy (Middle MW)
- **MW<1500**, RT

• WG-1241-7 (600 MHz grade, $10)
• WG-1228-7 (400 MHz grade, $9)

High-Throughput (low MW)
• up to 600 MHz, **MW<250**, RT
• WG-1000 ($2)



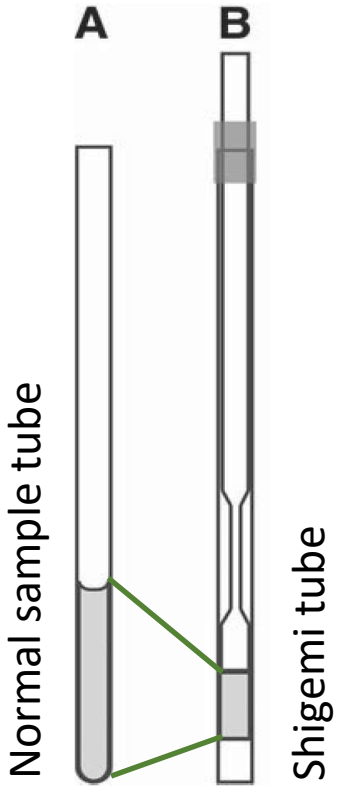


5mm Thin-Wall NMR Tube Comparison Table

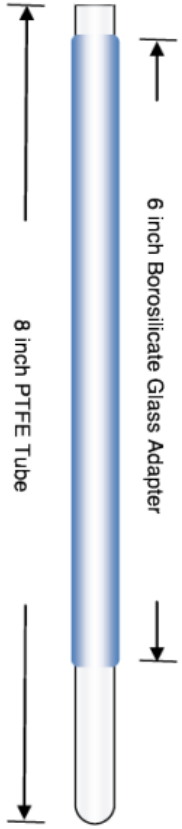
	High-Throughput	Economy	Precision (Glass)	Precision (Quartz)	Precision (Suprasil)
Material	Type 1 Class B Borosilicate Glass	Type 1 Class B Borosilicate Glass	Type 1 Class A Borosilicate Glass	Clear Fused Quartz	Synthetic Quartz
Impact On Shimming Quality By Paramagnetic Impurities	Medium (1200ppm Fe_2O_3)	Medium (1200ppm Fe_2O_3)	Small (400ppm Fe_2O_3)	None (0.5ppm Fe_2O_3)	None (<0.005ppm Fe_2O_3)
Maximum Working Temperature	Ambient	Ambient	230° C	1300° C	1300° C
Sample Volume Reproducibility	10%	10%	0.5%	0.5%	0.5%
Recommended Applications	Small molecule experiments up to 600 MHz (MW<250)	1D NMR experiments with small organic molecules (MW<1500)	Experiments requiring critical shimming quality (high-field, multi-dimension, multi-nuclei)	^{11}B NMR, rapid cooling/heating experiments, photochemistry studies	Photochemistry studies with deep UV light source

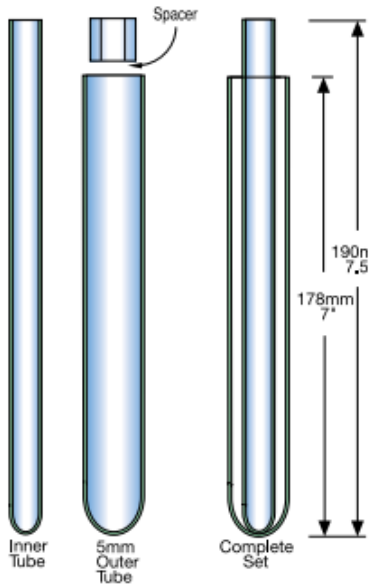
https://www.rototec-spintec.com/flyer/wilmad/NMR_Catalog_2020.pdf

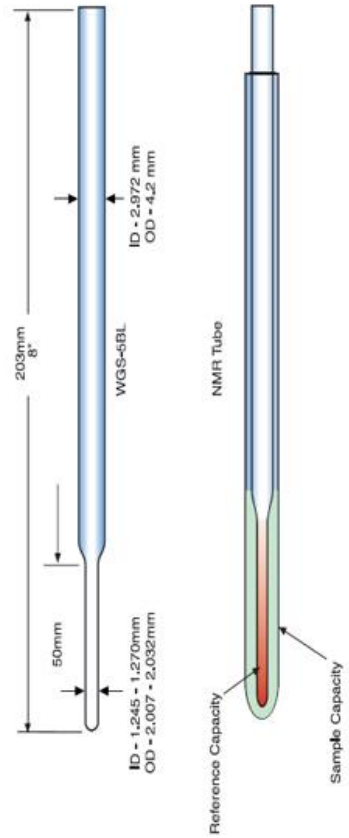
Special purpose sample tube



Reduce sample volume (1/3)







Shigemi tube
$ 300 - 400
- Increase 16% SNR
- Low con. or low volume

PTFE tube
$ 60
- HF or 29Si NMR

Coaxial tube
$ 40 – 50 (+ $ 50)
- Non-deuterated sample
- Quantitative NMR
- Referencing

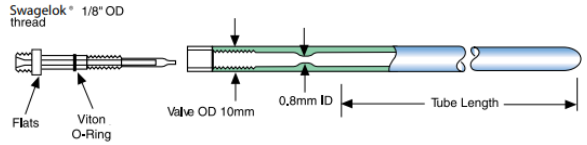
Stem Coaxial tube
$ 40 – 50 (+ $ 50)
- Non-deuterated sample
- Quantitative NMR
- Referencing

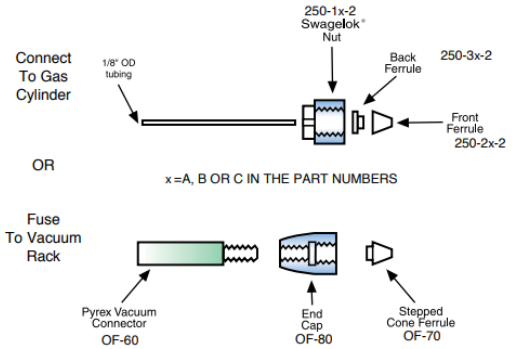
https://www.rototec-spintec.com/flyer/wilmad/NMR_Catalog_2020.pdf

Special purpose tube & cap

Pressure/Vacuum Sample Tube

Wilmad's Pressure/Vacuum Tube is the most reliable NMR tube for medium range pressure (<300 psi) experiments in the market. It is designed to connect to a 1/8" metal (stainless steel or brass) vacuum line using SwageLok® fittings or a rubber vacuum hose and a glass connector (OF-60). The PV-ANV valve is made of PTFE and all other parts are Pyrex® or equivalent glass. Valve is opened simply by turning counterclockwise.





Each Pressure/Vacuum tube is supplied with a PV-ANV valve, but not with a Swagelok® nut or ferrules. Order these separately (see connectors table).

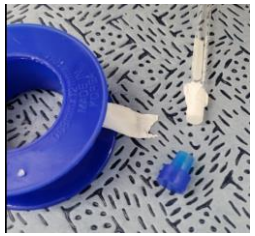
Low Pressure/Vacuum Tubes

Wilmad's Low Pressure/Vacuum (LPV) tube is ideal for anaerobic and gas-tight NMR experiments, and offers a convenient flame-free sealing solution for air sensitive or volatile liquid samples.

- Robust sealing system allows pressure build-up inside the sample
- Greaseless PTFE piston provides a 100% contamination-free seal
- Redesigned with a 4X larger sealing surface; eliminates leaks and greatly increases lifetime when compared to traditional J. Young tubes
- Axial symmetric design guarantees application in spinning experiments
- Due to the nature of glass, Extreme Caution should be exercised when using at elevated or reduced pressures since a tiny scratch on the glass surface would significantly lower the tensile strength. Adequate safety shielding should always be used when working in these conditions.

https://www.rototec-spintec.com/flyer/wilmad/NMR_Catalog_2020.pdf

Ethyl Vinyl Acetate Cap
- Cheap
- Avoid CDCl3, Acetone-D6



Ethyl Vinyl Acetate Cap
+ Teflon tape
- Cheap
- Use CDCl3, Acetone-D6

PTFE Cap
$ 16 (or $ 5 economy ver.)
- Use CDCl3, Acetone-D6, HF

Consumables (Recommendation)

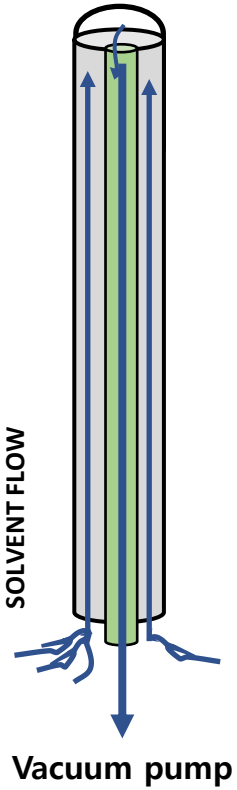
NMR sample tube (rating ≤ 600 MHz)

WG-1000-7 (5mm High Throughput NMR tube)
WG-1003-7 (Large writing Area 5mm High Throughput NMR tube)

Labeling tip
Short term: Red
Long term: Black

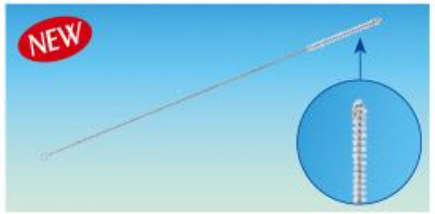
Tube washer

(Multi-tube Jet Solvent Wash/Dryer)



NMR pipet – Long tip pipette (13.75 inch)

NMR tube Brush



DURAN® NMR Tube Brush, Suitable for Φ5mm NMR Tubes, L250mm NEW
Made of Stainless-steel Wire & Nylon Bristles, <Germany-made>, Φ5mm NMR, 튜브용 브러쉬

* Curved End of Bristle Trim for Cleaning the Inner Tube Bottom
* Ideal for Cleaning NMR Tubes from Stubborn Contamination

<L1>

		Ea.	Price, ₩
DU.2907400	Brush, for Φ5mm NMR Tubes, SS Wire w/Nylon Bristles, L250mm		

Volume and concentration

• Sample volume = 500 ul (400 ~ 700 ul)

• Sample concentration

1. 1H: 0.05 mM ~ 10 mM (Too high concentration cause Line-broadening effect)

2. 13C, 2D NMR(COSY, NOESY, TOCSY, etc.): 10 mM ~ 100 mM

3. 15N, 17O, Hetero 2D NMR (HSQC, HMBC, HMQC, etc.): 100 mM < Highest concentration

ex) Sample A (MW=200), 0.05 mg / 500 ul = 0.5 mM = 100 ppm

Sample A (MW=200), 1 mg / 500 ul = 10 mM = 2000 ppm

Sample B (MW=500), 25 mg / 500 ul = 100 mM

Referencing

<Internal reference>

1. Sample + NMR solvent (CDCl3, D2O, DMSO-D6, etc.) + reference compound (TMS, DSS)

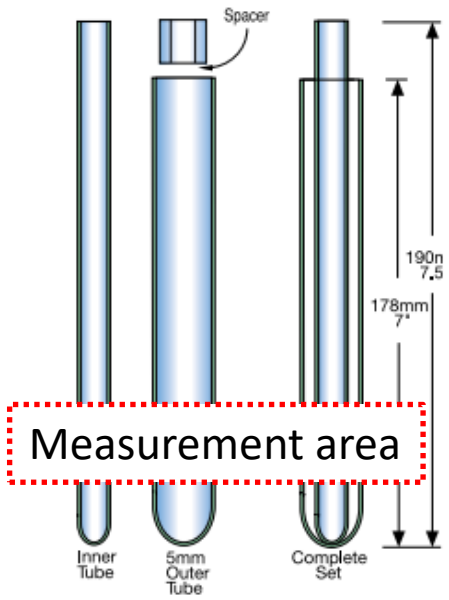
<External reference>

2-1. Sample + NMR solvent (CDCl3, D2O, DMSO-D6, etc.)

2-2. Reference sample (P31: TPP = -17.57 ppm, F19: TFT: -63.72 ppm)

3. Coaxial sample tube (Evans method)

- Inner tube: NMR solvent or Reference
- Outer tube: Sample



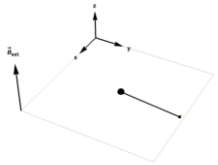
- None deuterated solvent condition -> No Lock / No auto shim -> Poor or not easy measurement
- NMR solvent and Referencing (+Quantitative NMR)

Paramagnetic effect

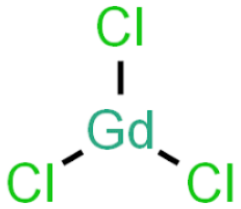
• Degassing

Oxygen (Paramagnetic) -> Short T1&T2 relaxation-> **Quench** for NMR signal -> Purge N2 or Ar gas

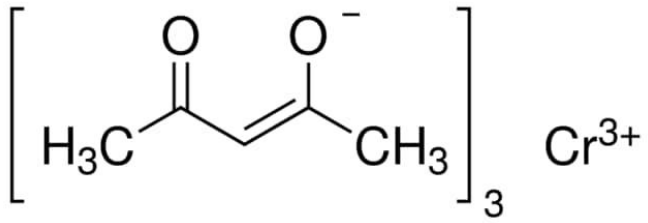
• Doping agent (Use Paramagnetic agent)



- Reduce T1 & T2 -> Short experiment time
- Line broadening effect (poor shimming)
- Add paramagnetic material concentration: 1 mM (1H), 25 mM – 50 mM (13C)



GdCl3
Gadolinium(III) chloride



Cr(acac)3
Chromium(III) acetylacetonate

CuSO4, MnCl2, NiCl2

Dielectric constant : Polar > 20 > Non-polar

https://onlinelibrary.wiley.com/doi/full/10.1002/masy.201300034

Sample preparation

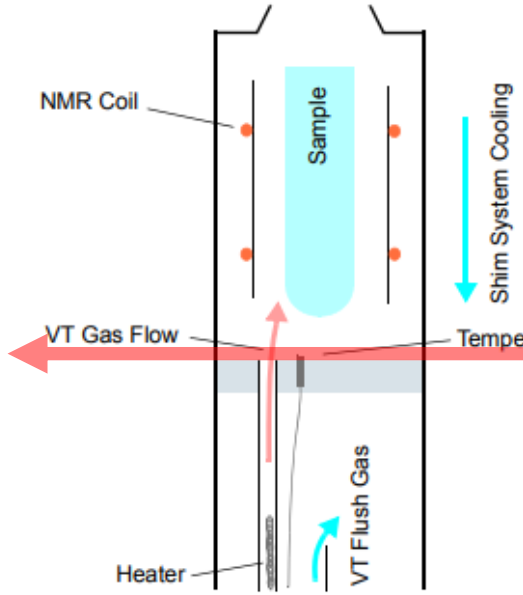
- Wash sample tube to acetone or ethanol.

  Cheap sample tube dry-situation tilted state in oven make possible bending

  (Sample tube bending test → rolls the ground If bending

sample tube spins, quartz glass in probe has some damage.)

Probe Bottom



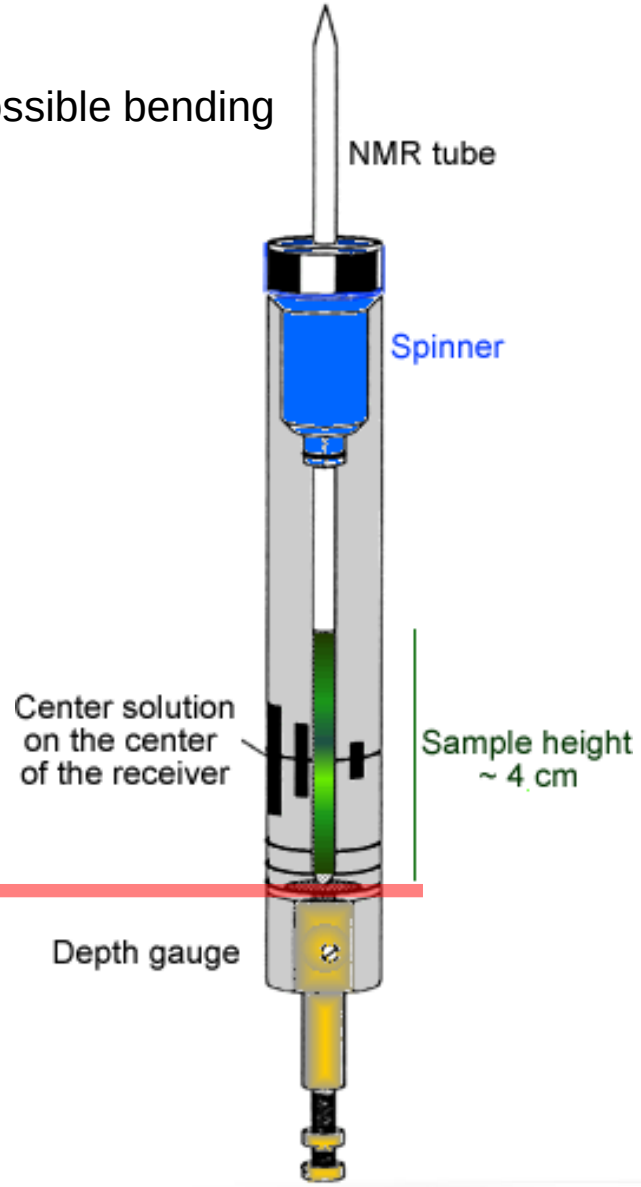
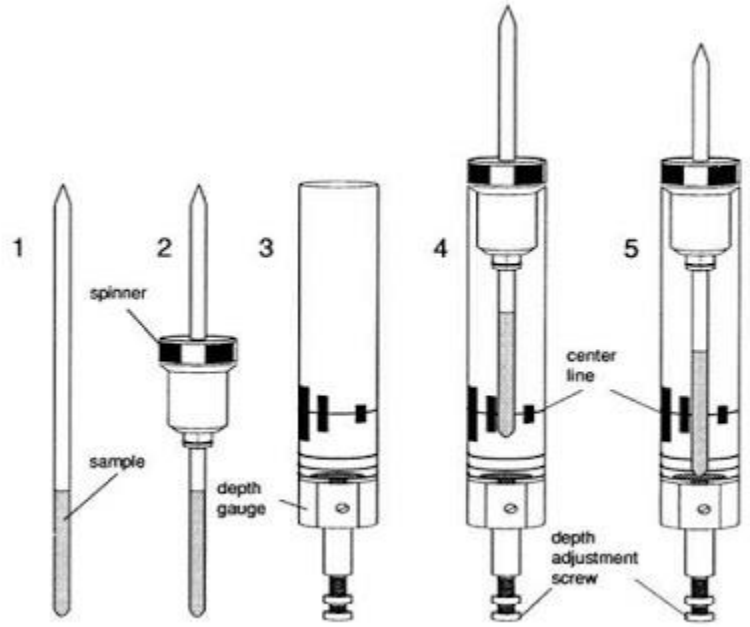


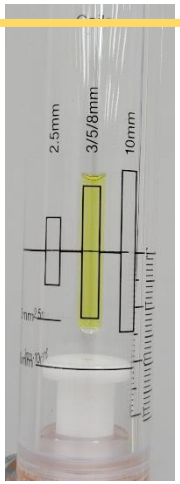
Figure 3.1 Sketch of sample temperature control in a NMR probe

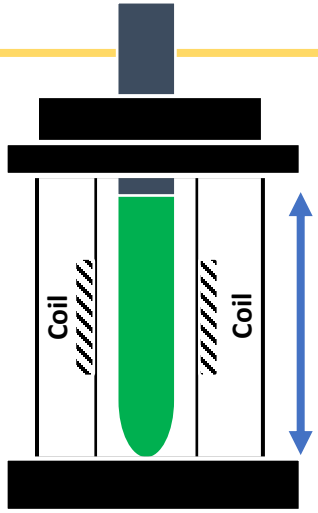
Sample preparation

1. Perfect solution sample filters syringe filter.
   (If your sample has insolubility, It makes low resolution and sensitivity about peak)
2. 5 mm sample tube inserts sample 0.5 ml (500 μl, 4cm)
3. Spinner placed on depth gauge insert sample tube
   (※ Don`t touch O-ring)
4. Check location depth gauge center (dash line square) with sample tube
5. Remove depth gauge.



Sample volume and height

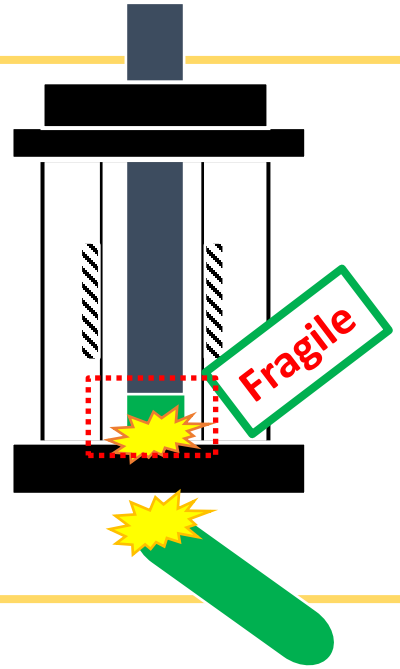




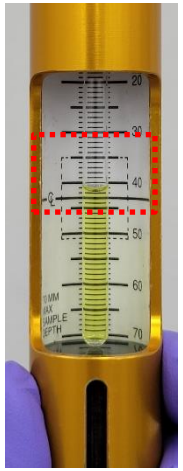
40 mm
400 – 500 uL

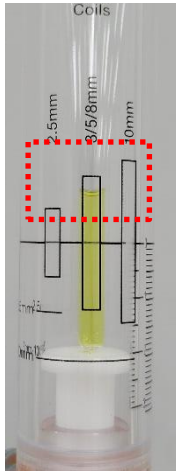
BEST

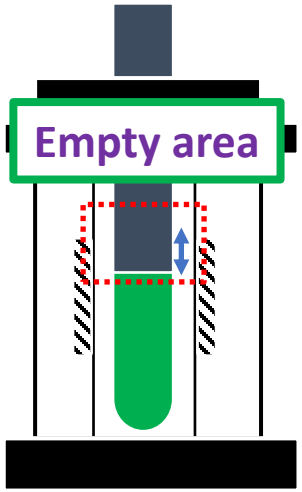
Worst



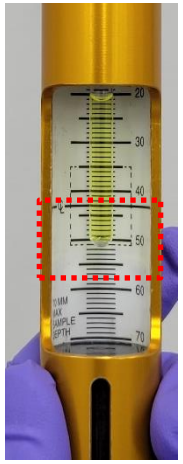
Bad shim

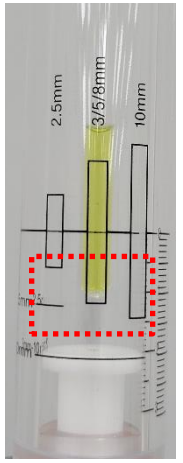


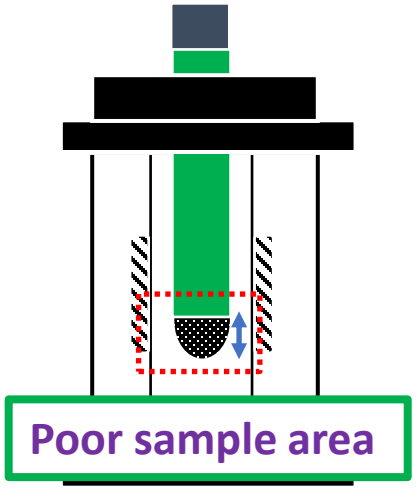




Bad shim

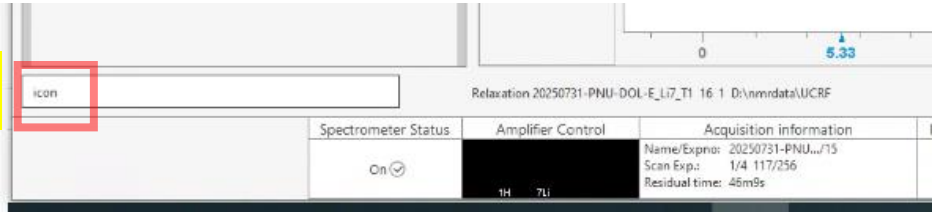




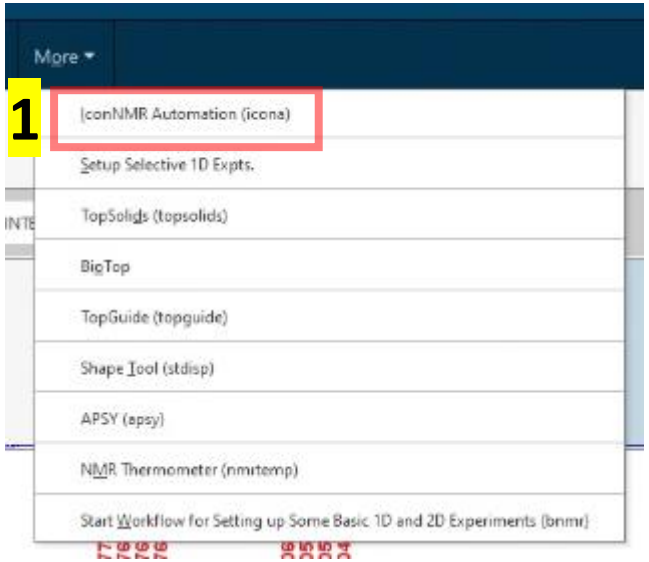


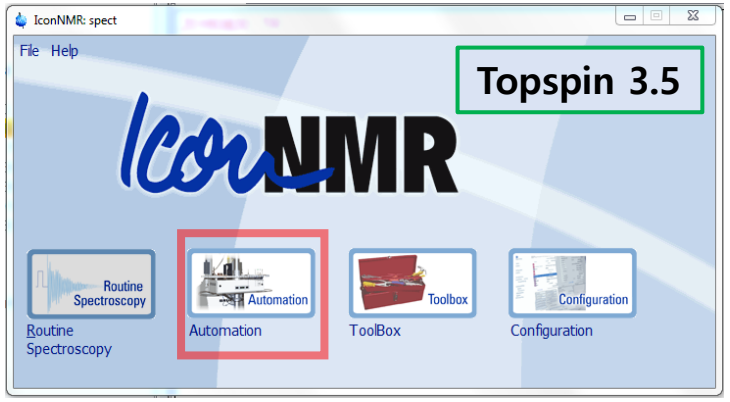
Operation With Icon-NMR

Icon-NMR (Login)

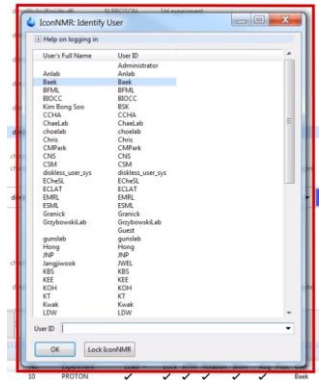


Type ***icon*** on command line
Click Acquire tab > More > IconNMR Automation





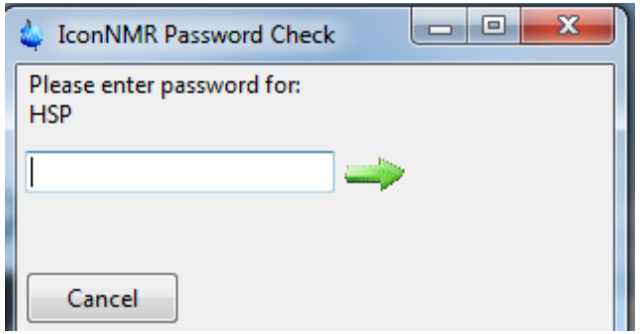
2 Click **Automation**



3 Select Lab ID

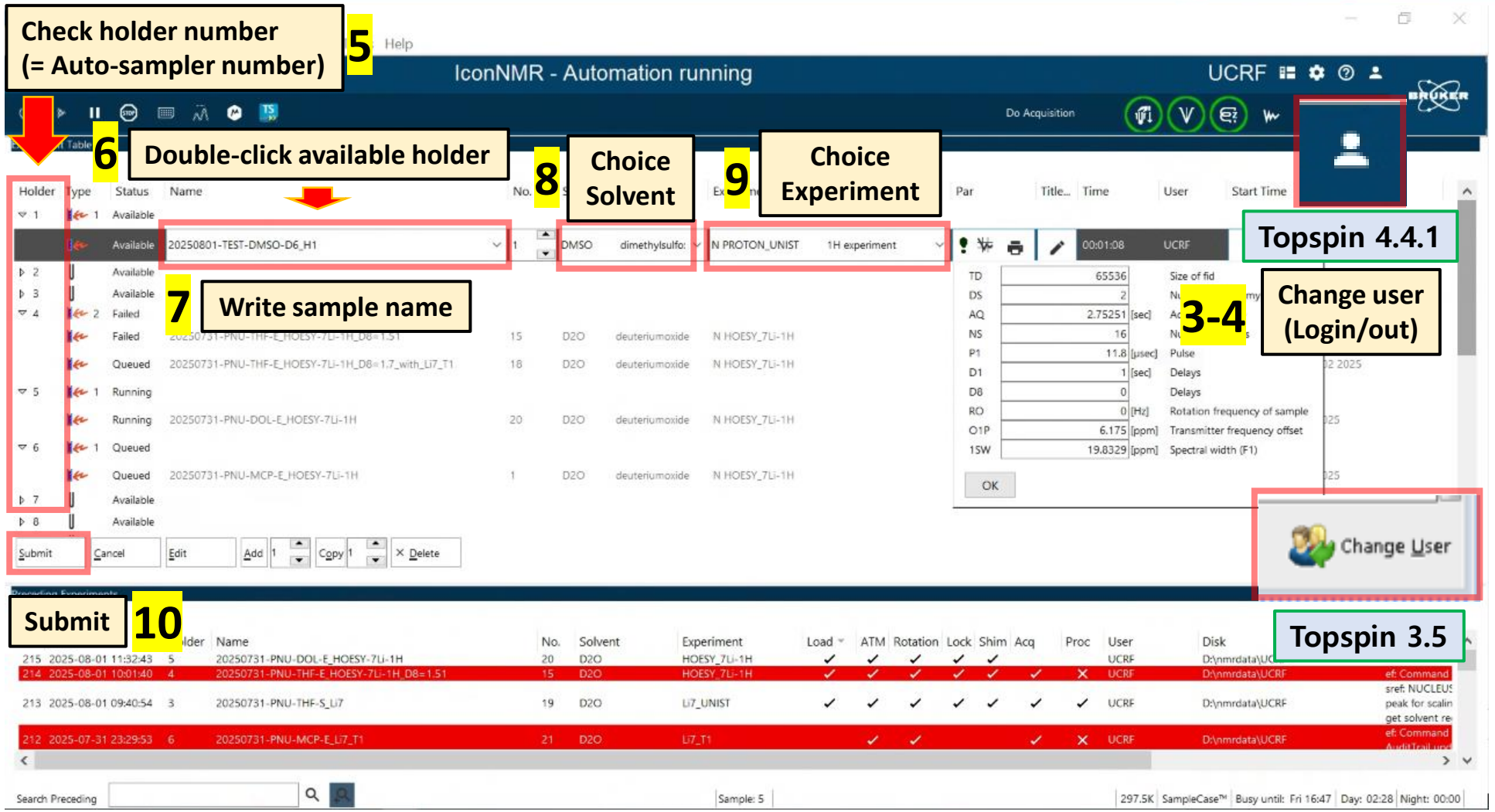
Topspin 3.5

Topspin 4.4.1

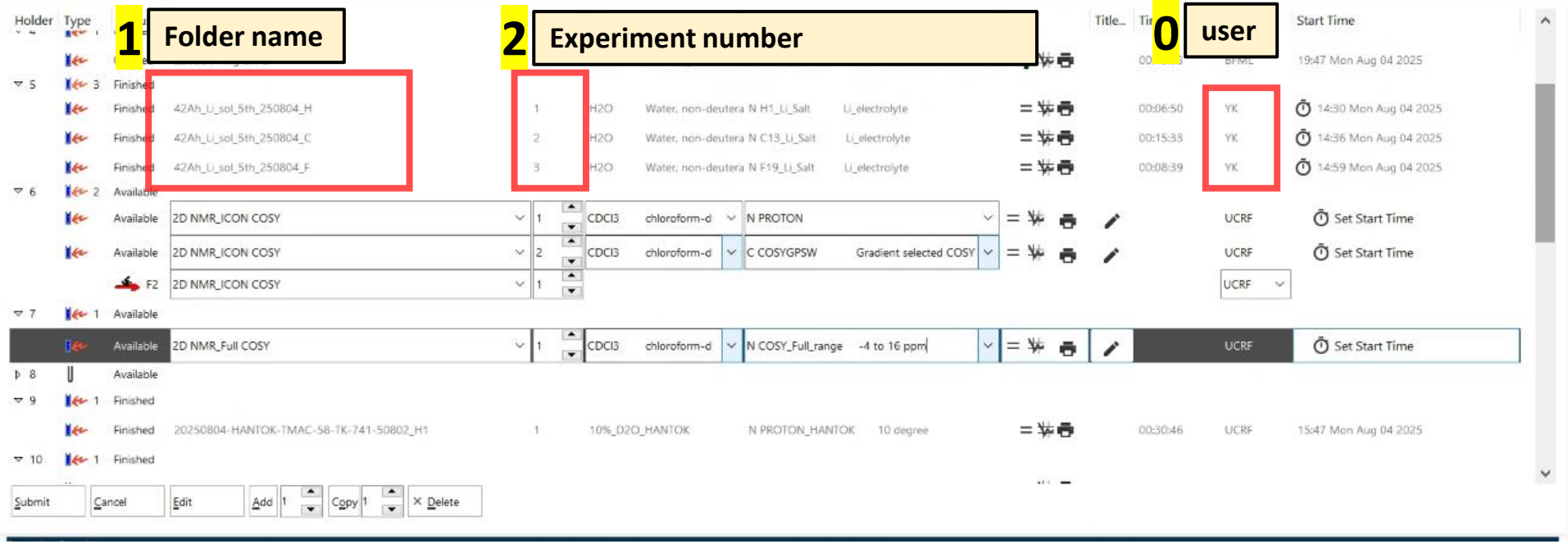


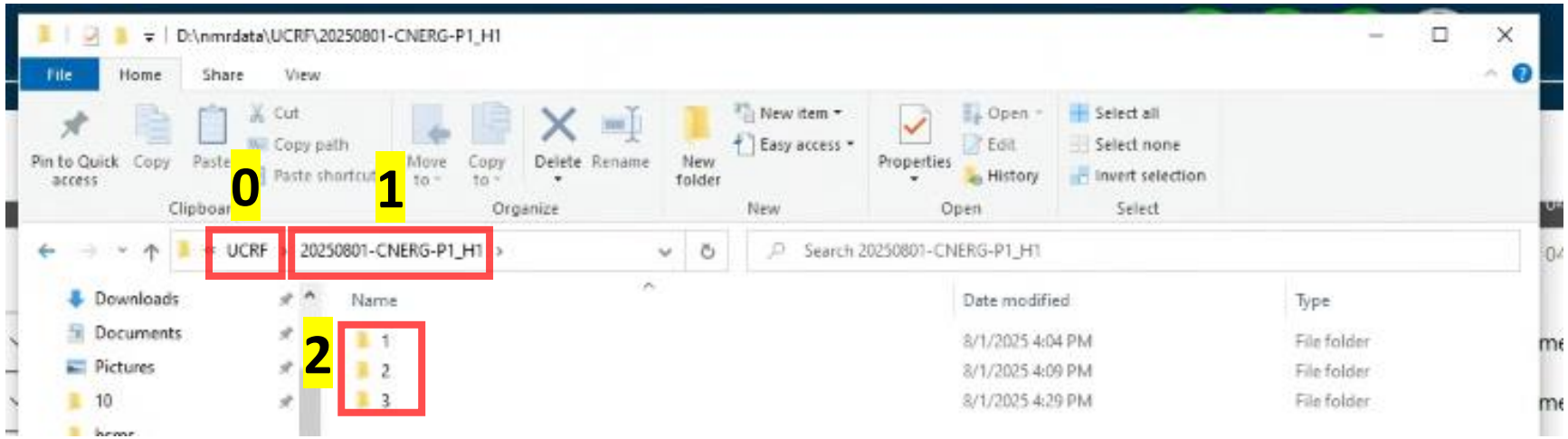
4 Type password

Icon-NMR (Routine)

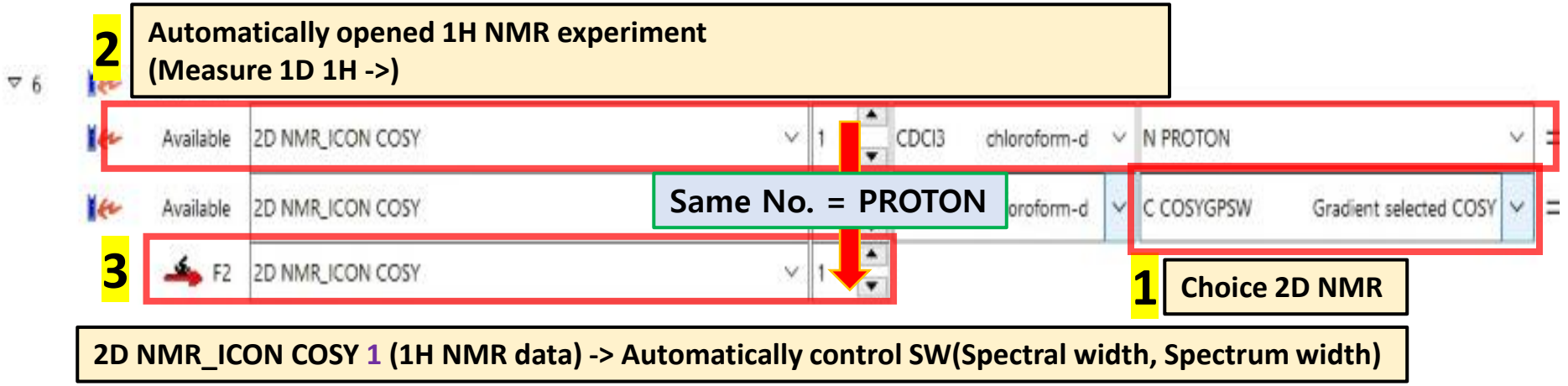


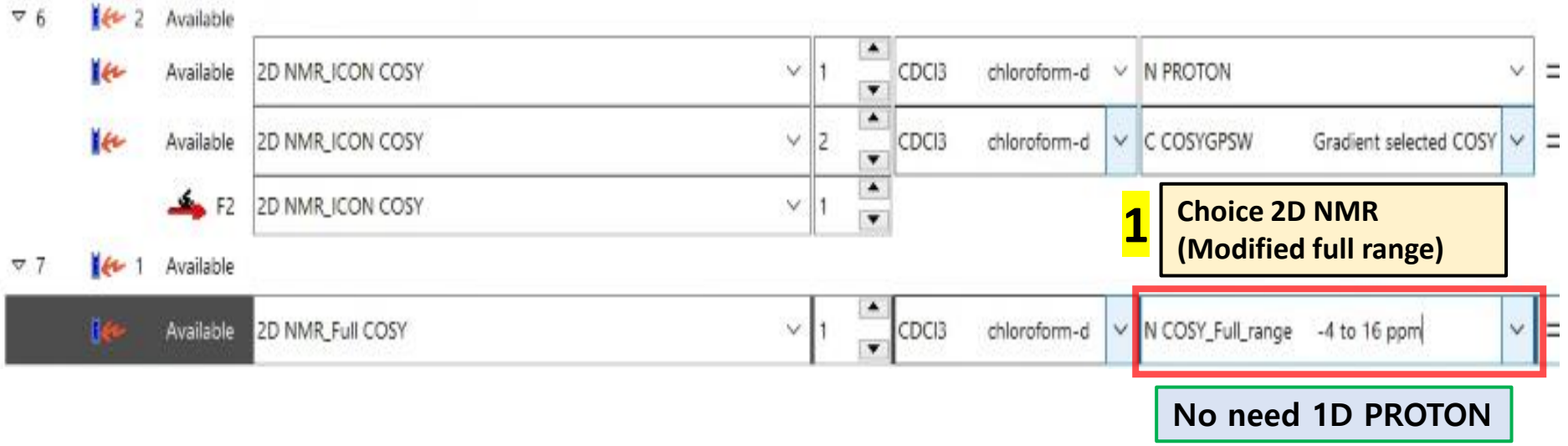
Icon-NMR (Folder name)



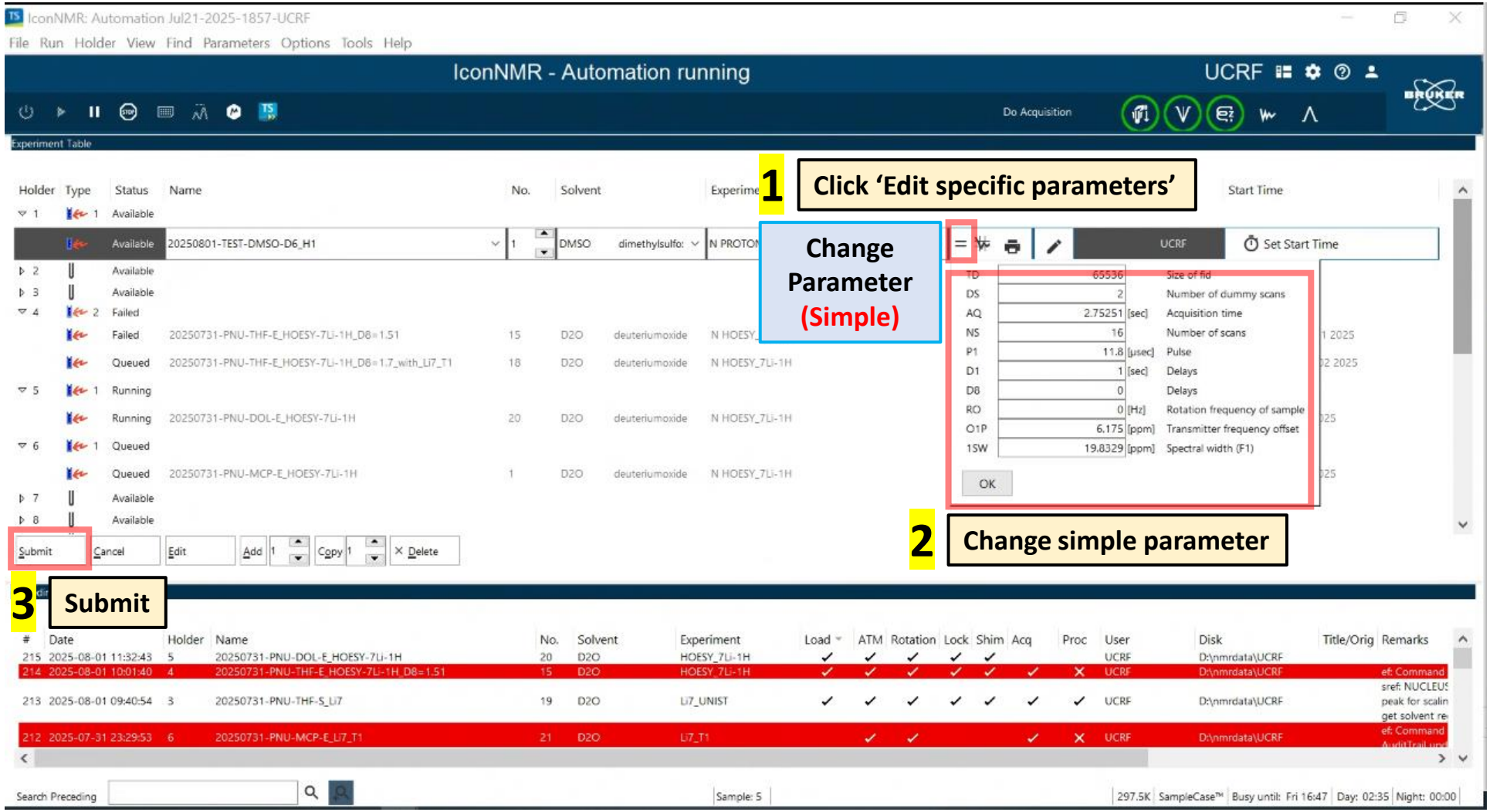


Icon-NMR (2D NMR)

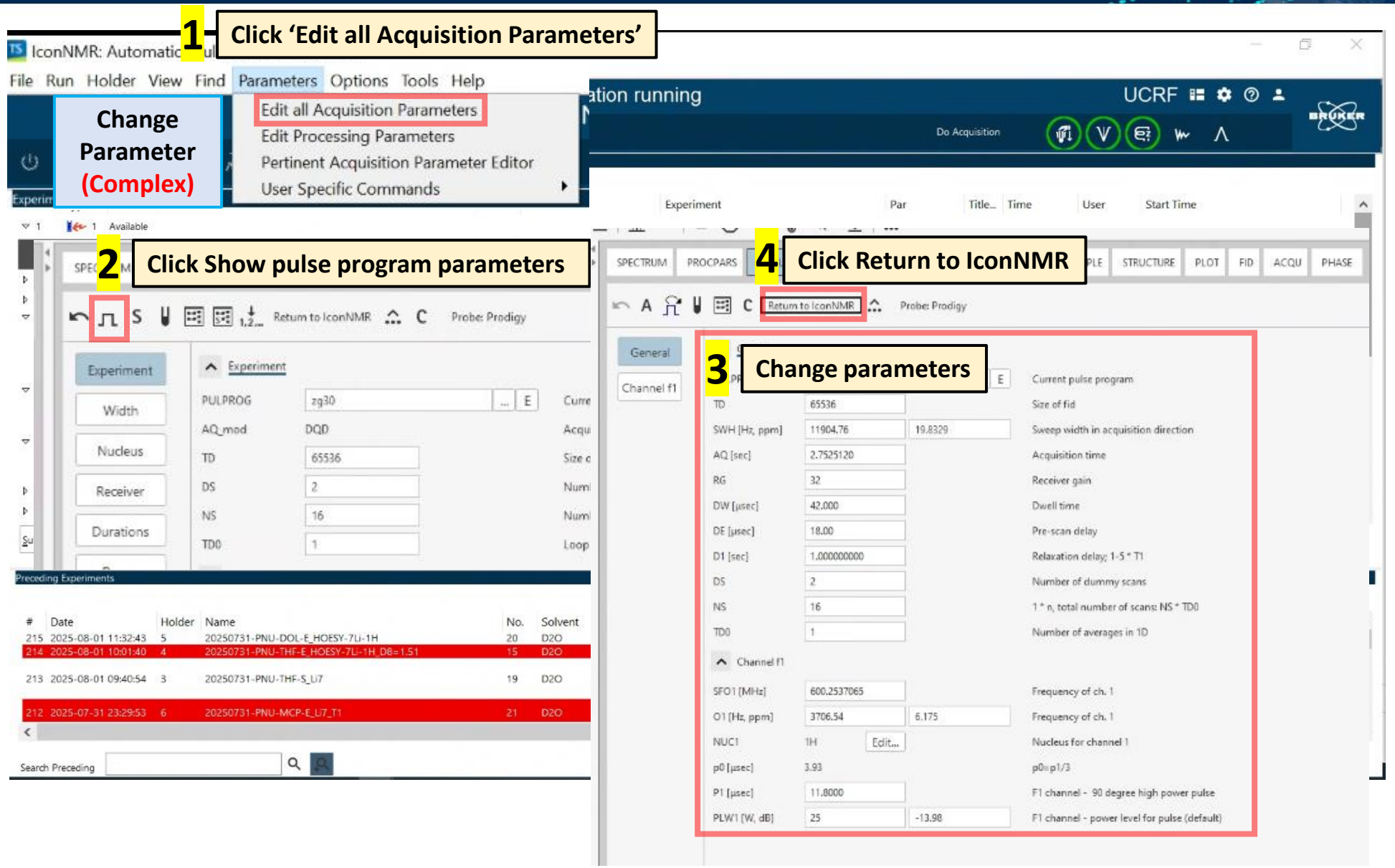




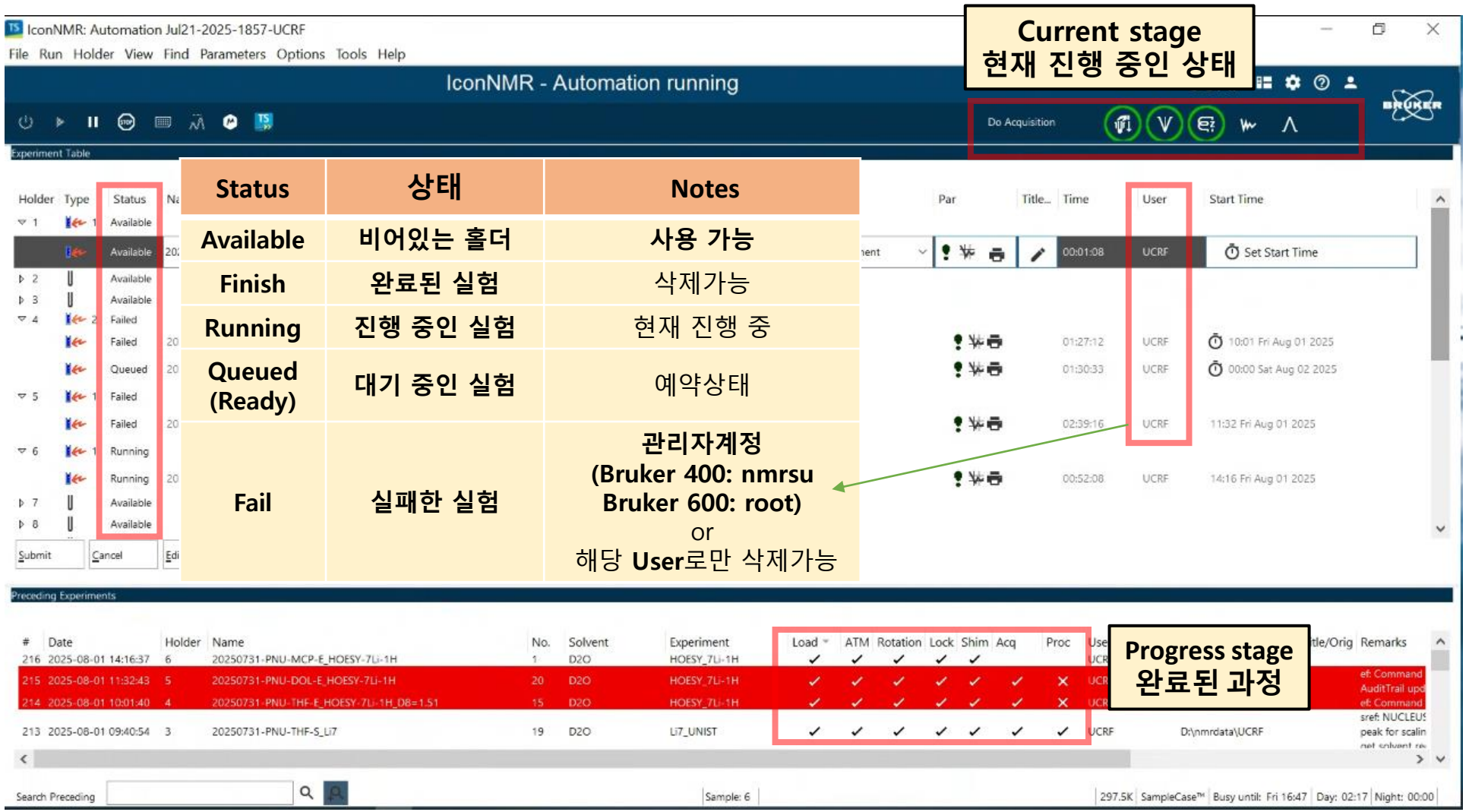
Icon-NMR (Change parameter - Simple)



Icon-NMR (Change parameter - Complex)



Icon-NMR (Status)

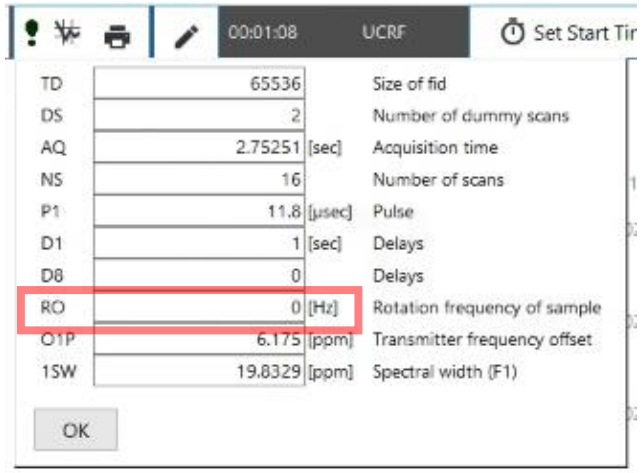


Status	상태	Notes
Available	**비어있는 홀더**	**사용 가능**
Finish	**완료된 실험**	삭제가능
Running	**진행 중인 실험**	현재 진행 중
Queued (Ready)	**대기 중인 실험**	예약상태
Fail	**실패한 실험**	**관리자계정 (Bruker 400: nmrsu Bruker 600: root)** or 해당 **User**로만 삭제가능

Icon-NMR (Button)

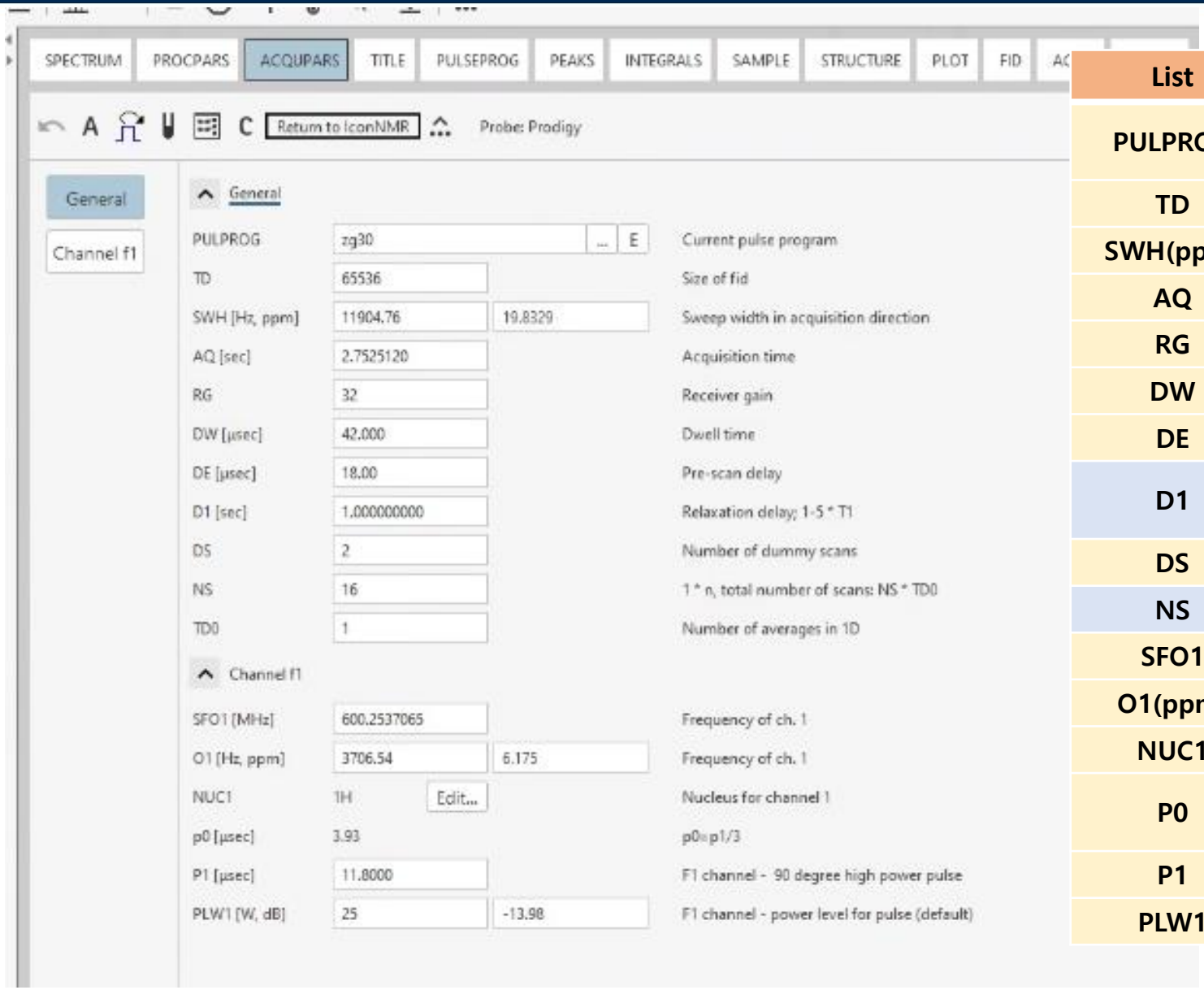
Button	Function	Notes
Submit	**Run experiments**	원하는 홀더 번호 선택 후 Submit
Cancel	**Queued -> Available**	준비 -> 사용가능상태로
Edit	**Available -> Edit**	수정필요시
Add	**1 Sample -> Multiple experiments**	Save time, Lock/Shim just run 1 time (시간절약, Lock/Shim은 1회만 진행)
Copy	**Copy Sample name, Solvent, Parameter (For Similar experiment)**	실험명, 용매, 실험, 파라미터 복사 (유사한 샘플 또는 유사한 실험 진행시 활용)
Delete	**Delete experiment**	실험삭제 (Fail 상태는 User or Admin만 삭제가능)

Icon-NMR (Spin on/off)



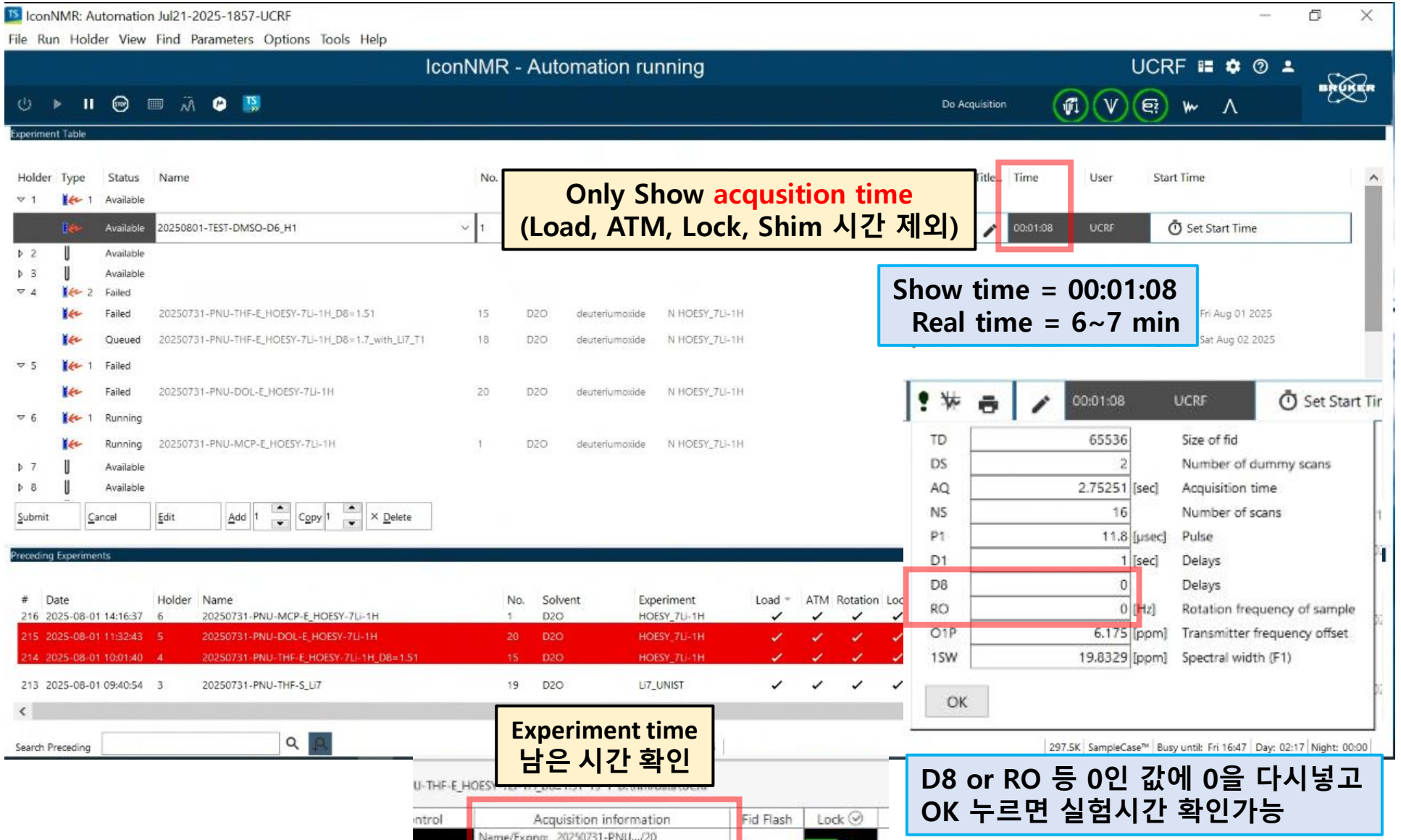
RO (Rotation On)
Spinning = 20
Non Spinning = 0

Icon-NMR (Parameter list)

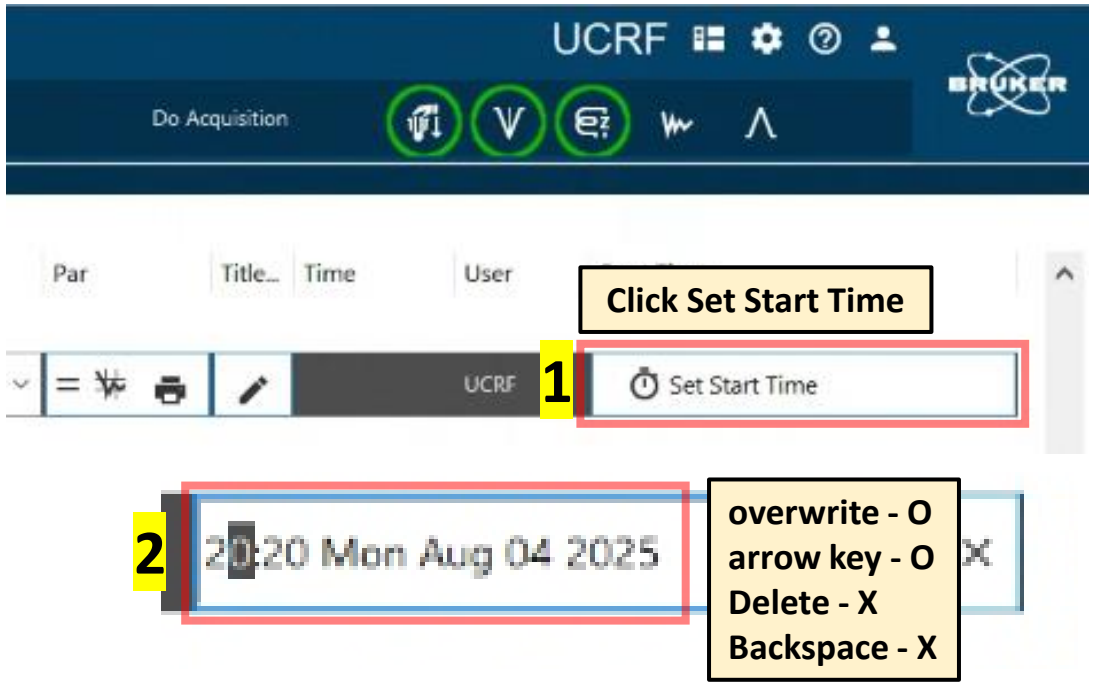


List	Explanation	Notes
PULPROG	**실험프로그램**	**zg30(30도 펄스) zg (90도 펄스)**
TD	Time domain	FID point 개수
SWH(ppm)	Spectral width	스팩트럼 총 폭 (X축)
AQ	Acqusition time	데이터 얻는 시간 (TD * DW)
RG	Reciever gain	Detector window size
DW	FID Point사이 간격	
DE	Acquisition 이전 delay	
D1	Relaxation delay time	Integration값과 연동 T1 * 5
DS	Dummy scan	실제스캔이전 준비운동
NS	Number of scans	실제스캔
SFO1	Resonace frequencsy	Target 핵종의 에너지 분리량
O1(ppm)	Center frequency	스팩트럼 중심값
NUC1	Target nucleus	측정하고자 하는 핵종
P0	Input RF pulse (time)	실제로 들어가는 RF pulse 시간 ex) zg30 (P0=P1/3)
P1	Input RF pulse (time)	입력된 RF pulse 시간
PLW1	RF power	RF pulse power level

Icon-NMR (Time)



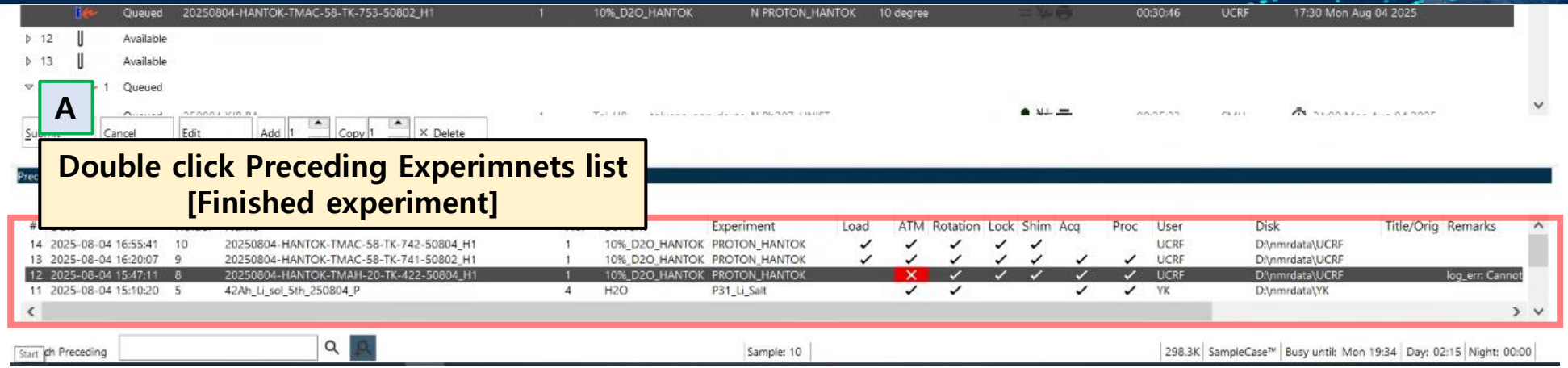
Icon-NMR (Reservation)

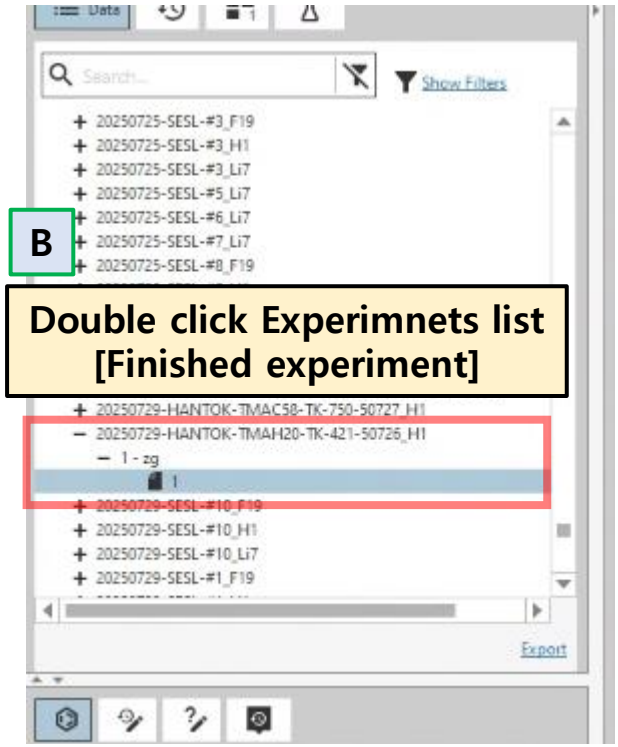


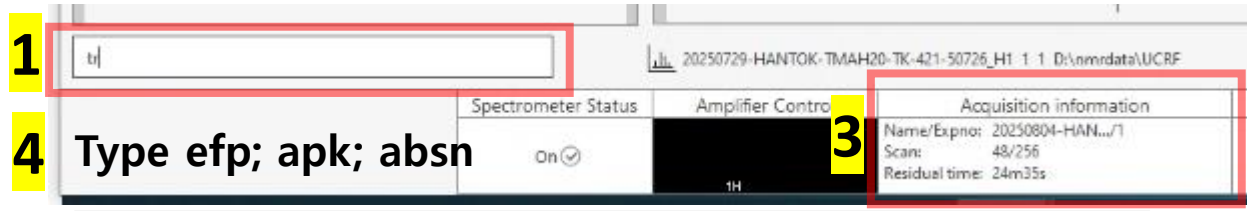
3 Many samples
Just +1 min is enough

14	1	Queued								
		Queued	250804 KJB BA	1	Tol-H8	toluene, non-deute	N Pb207_UNIST	00:25:23	SMH	21:00 Mon Aug 04 2025
15	1	Queued								
		Queued	250804 KJB DPP	1	Tol-H8	toluene, non-deute	N Pb207_UNIST	00:25:23	SMH	21:01 Mon Aug 04 2025
16	1	Queued								
		Queued	250804 KJB BSA	1	Tol-H8	toluene, non-deute	N Pb207_UNIST	00:25:23	SMH	21:02 Mon Aug 04 2025

Icon-NMR (Open data)





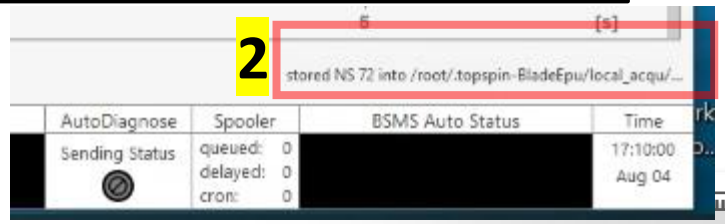


C

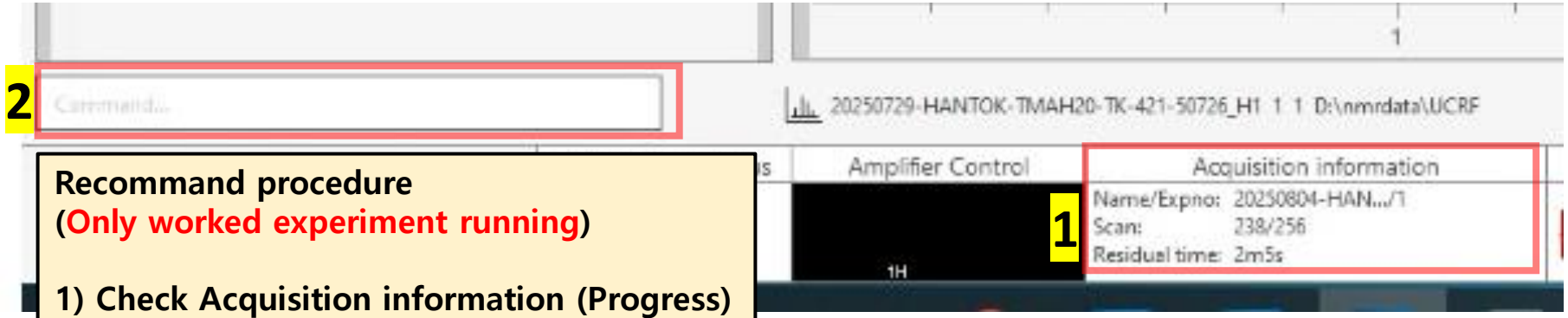
Save & show data in progess

1) Type *tr* on command line
2) Check Stored NS OO
3) Double click Acquisition information
4) Click Process Proc. Spectrum (or Type efp; apk; absn)

efp: FID to spectrum
apk: Auto phase correction
absn: Auto baseline correction

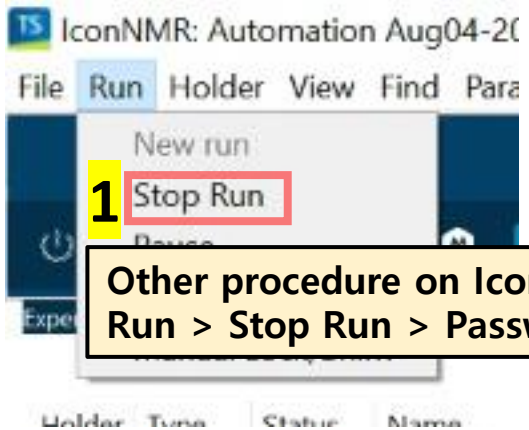


Icon-NMR (Stop run)

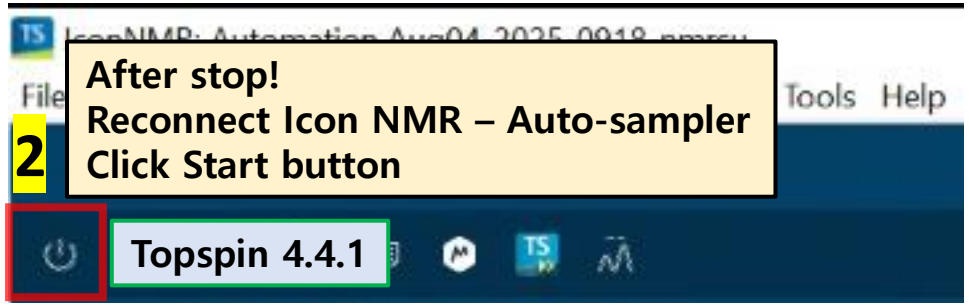


Recommand procedure
(Only worked experiment running)

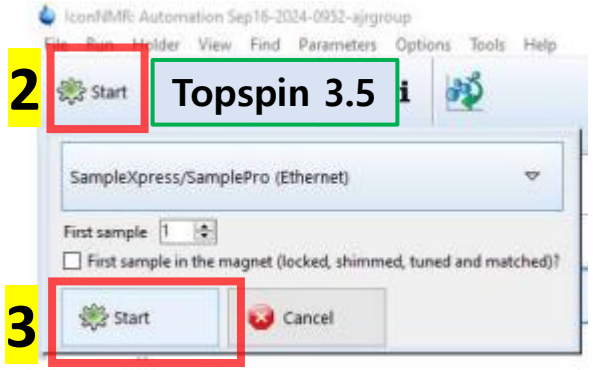
1) Check Acquisition information (Progress)
2) Type *halt* on command line
***halt* : Save and stop**



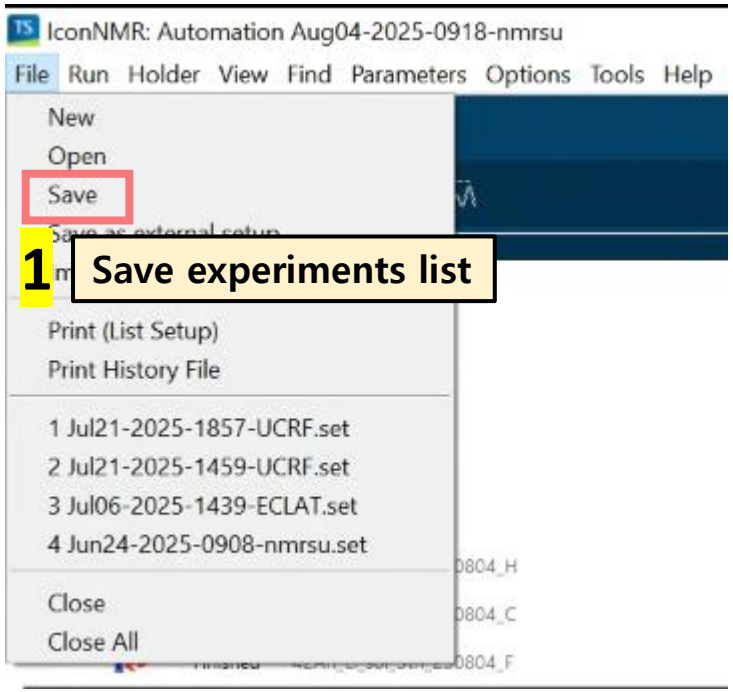
Other procedure on Icon-NMR
Run > Stop Run > Password: bruker

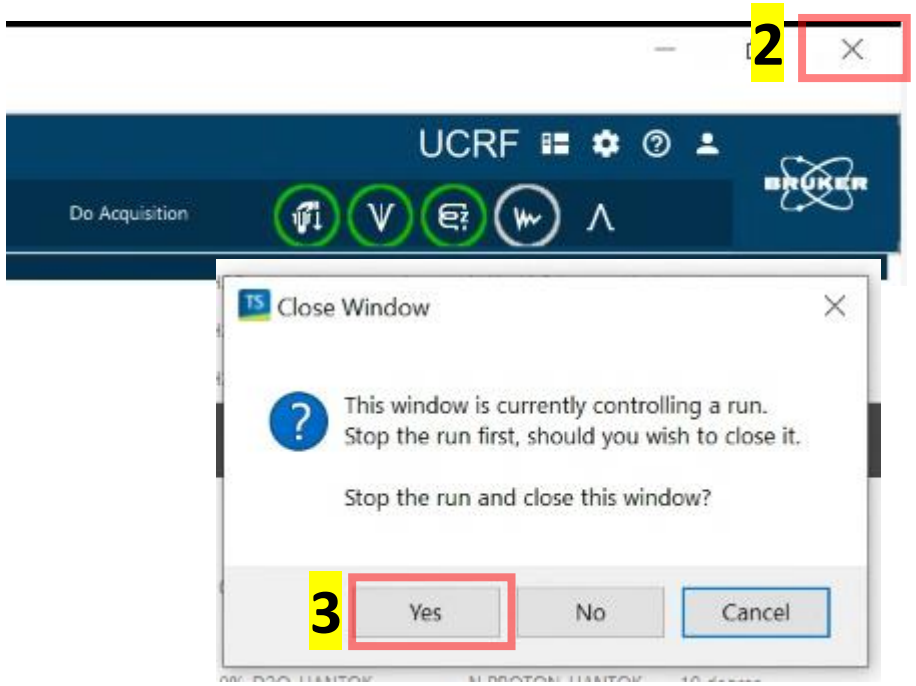


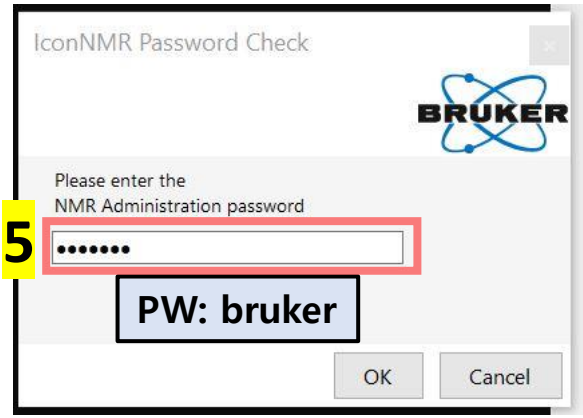
After stop!
Reconnect Icon NMR – Auto-sampler
Click Start button



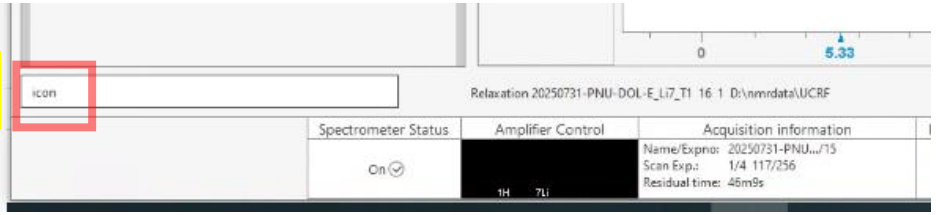
Icon-NMR (Turn off)



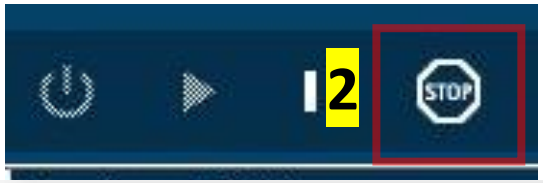


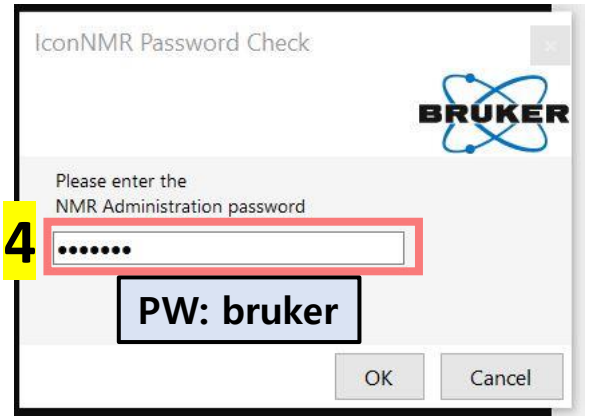


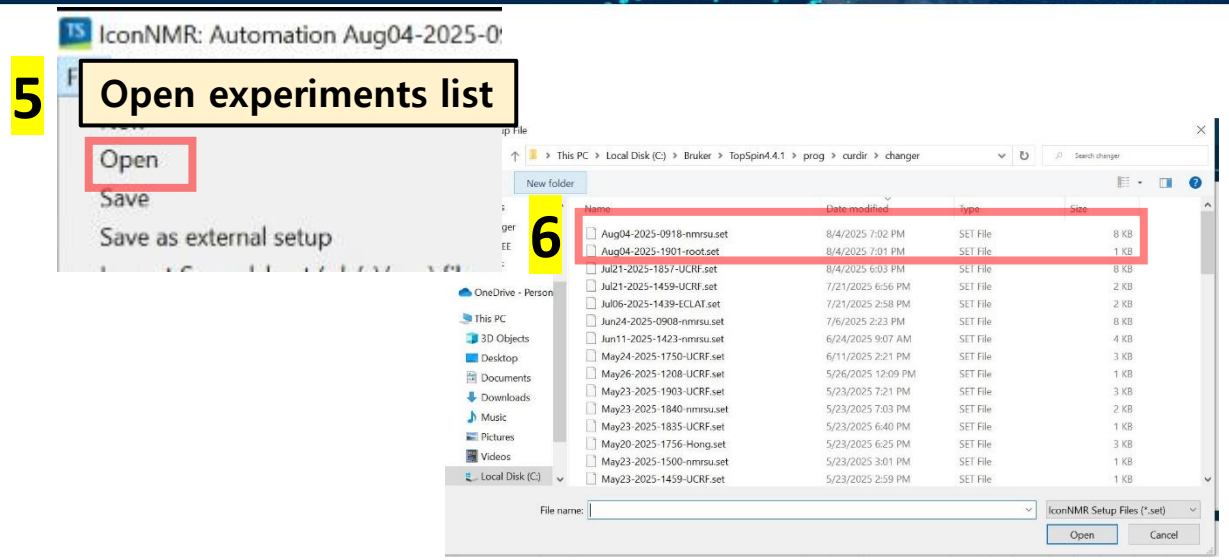
Icon-NMR (Turn on)

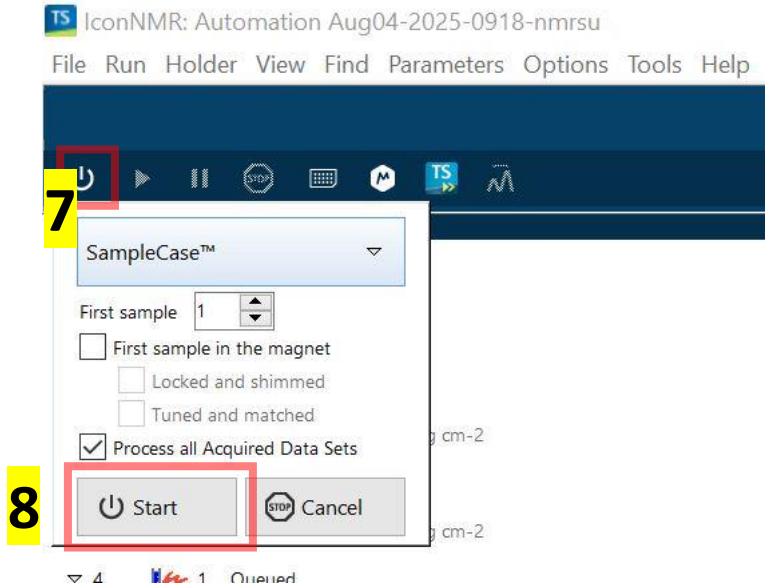


Type ***icon*** on command line









Icon-NMR (Admin)

Bruker 600
Software: Topspin 4.4.1
Windows10

Admin (Icon-NMR)
ID: root
PW: topspin

Windows 10
ID: nmrsu
PW: topspin

Topspin/Icon-NMR config
PW: bruker

Admin (Icon-NMR)

1) Delete fail experiment
2) Exit Icon-NMR
3) Control other user list

Windows 7/10

1) Turn on PC

Topspin/Icon-NMR config

1) Exit icon-NMR
2) Change configuration

Bruker 400
Software: Topspin 3.5 pl7
Windows7

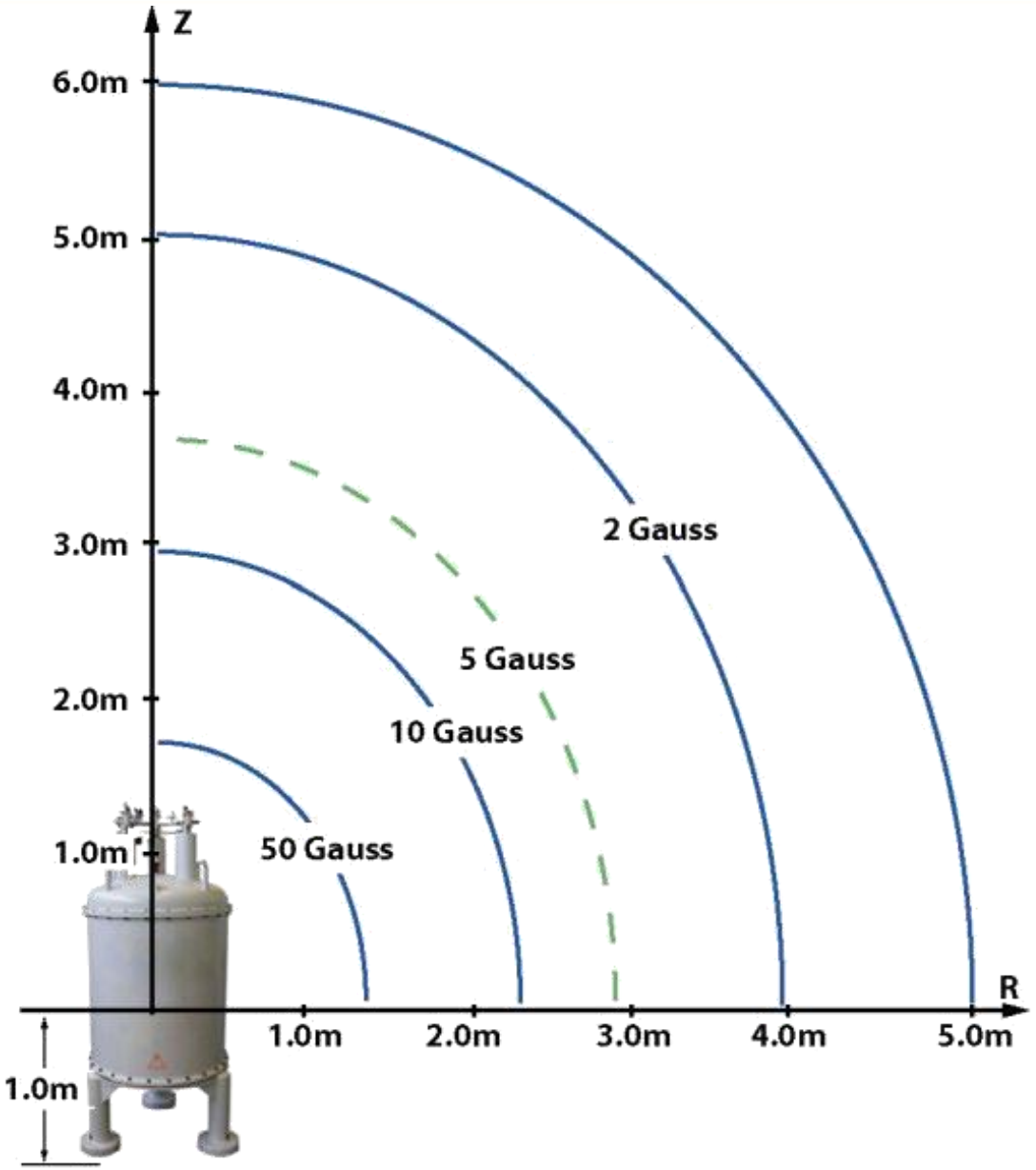
Admin (Icon-NMR)
ID: nmrsu
PW: topspin

Windows 7
ID: nmrsu
PW: topspin

Topspin/Icon-NMR config
PW: bruker

Safety

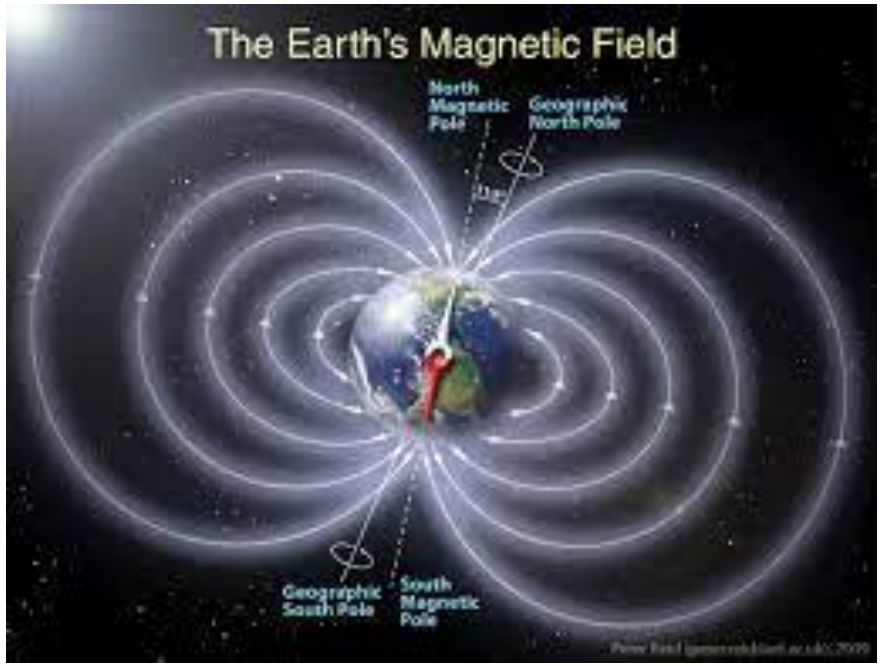
5 Gauss line



5 Gauss line

'Safe' levels of static magnetic field exposure for general purpose

Earth`s Magnetic field = 0.5 gauss



Warning signs

Caution: Extremely high magnetic stray fields.

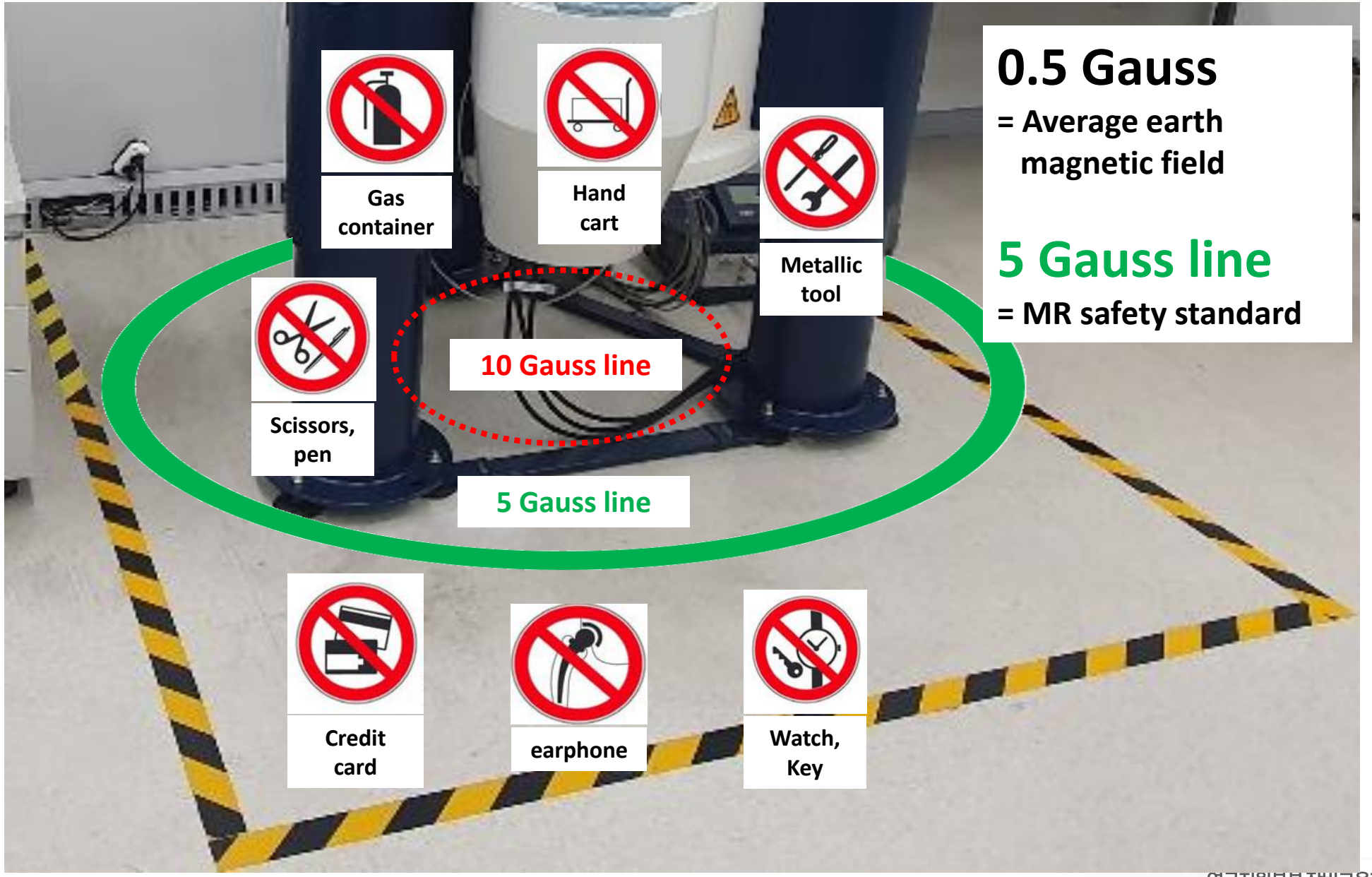
Danger: No entrance for people carrying pace-makers.

Danger: No entrance for people carrying medical implants.

Caution: Watches and electronic or electro-mechanical devices may be damaged.

Caution: Credit cards, magnetic storage media as tapes, floppy disks or hard disks, may be damaged.

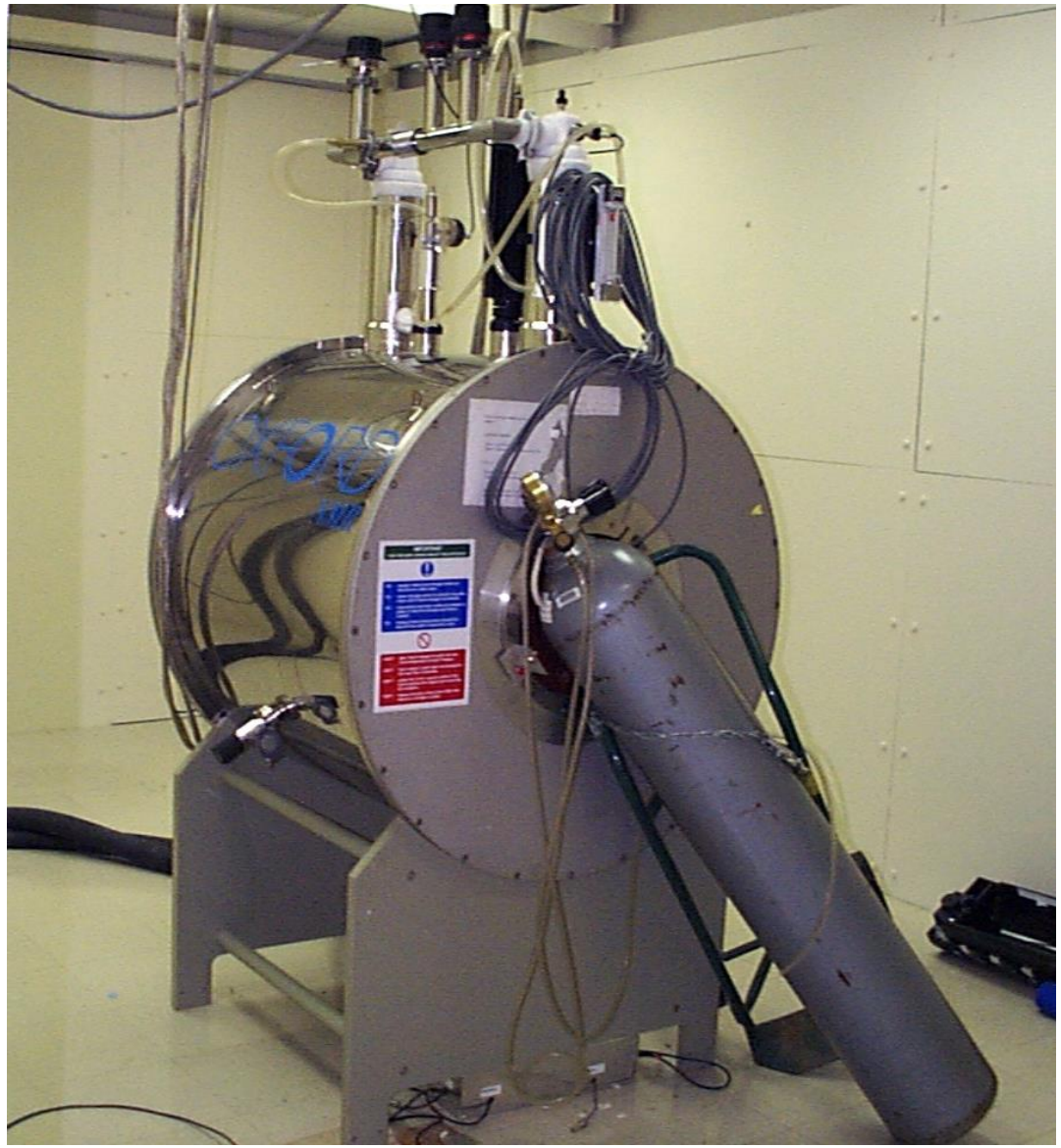
Warning signs



0.5 Gauss
= Average earth magnetic field

5 Gauss line
= MR safety standard

Magnetic safety



Magnetic safety

Human Body react to Oxygen-Deficiency

O2 Concentration 21%

Symptoms

Natural air

O2 Concentration 18%

Symptoms

Limit level for not causing serious health problems. Continuous ventilation is required

O2 Concentration 16%-12%

Symptoms

Rapid breathing, Increase in pulse rate, Loss of concentration, Headache, Nausea, Ear ringing

O2 Concentration 14%-9%

Symptoms

Stupor, Headache, Nausea, Cyanosis, Faintness on the entire body

O2 Concentration 10%-6%

Symptoms

Comatose, Loss of consciousness, Muscle spasm on the entire body

O2 Concentration 6% or less

Symptoms

Unconsciousness, Comatose, Cessation of breathing, Cardiac arrest, Die in 6 minutes

Magnet quench

https://youtu.be/d-G3Kg-7n_M?t=5