Avance III 500 Manual Use Procedures

The typing of commands is done in the box at the bottom of the spectrum, and is completed by an enter/return key. Some mouse operations cause the command box to become inactive, move the cursor to the command box and left click to reactivate it. Often the staff uses typed commands to perform functions (and there are some that must be done by command entry), but most commands are available in the gui menus and directions for their use are included.

Most of the instrument controls, as opposed to graphics control functions, are found in the tabs at the top of the screen labeled Acquire, Process, Analyze, Applications, and Manage. Each opens a set of functions related to the heading. Graphics control functions are present in buttons at the top of the data window. Bruker numerical parameters like ns (number of scans), o1 (center of spectrum), etc. can be changed by typing ns <#>, which sets the new value, or ns <enter>, which opens a small pop-up that shows the present value and it may be adjusted.

Safety Notices

- Never, for any reason, are you to bring a person with a pacemaker close to a magnet.
- Avoid bringing ferromagnetic materials anywhere near a magnet. This includes most tools and office supplies.
- Be wary of bringing electronics close to a magnet, we are not responsible for their loss.
- Do not use the instrument for any purpose other than those for which it has been designed and set up.
- If you do not hear the cold head running, do not attempt to use the system.
- Do not bring food or drink into the lab.
- Do not leave the lab door open for extended periods, the system is sensitive to water.

Sample Preparation

Samples should be prepared in high-quality 3 mm or 5 mm NMR tubes; for example: Wilmad 528 PP, which are sold in the Chemistry Stockroom. **Do not use lower-quality (inexpensive) NMR tubes**, these inevitably produce poor results and could even damage the probe. Sample should be 0.11-0.12 mL for 3 mm tubes or 0.5-0.6 mL for the 5 mm tubes, and no less. Additional solvent does not greatly hurt instrument performance, but it does dilute the sample and should be avoided if the target quantity is small. Use new, high quality, dry, and deuterated NMR solvents, and avoid solvent contact with plastic materials (pipettes, bottles, eppendorf tubes, et cetera) as they can leach organic materials into your sample.

Login Procedures

There are two types of users on this system. Users that are not trained to use the system in manual mode will only have a login to the software, which allows them to run the system using IconNMR. **We strongly prefer that experiments be done in IconNMR if possible**, but there are circumstances in which this is not possible. For those users that require running the system outside of IconNMR, they will also be able login for the computer itself. The majority of procedures outlined in this document are for those running the system manually; but, regardless, please remember these Rules and Etiquette:

- Never, for any reason, allow anyone else to use your login information. Violating this rule can have severe repercussions at the university level for all involved.
- Do not make a reservation for anyone else or that you do not plan to keep, and delete a reservation if circumstances prevent you from keeping it as planned.

- Never take someone else's sample out of the sample changer.
- Your experiments are expected to be completed within your reserved time.
- You may use the instrument without a reservation, but if someone has made a reservation during a time slot: that person has priority and you must surrender the instrument to that person.
- When your immediate use for the instrument is done you should log out. Never power down the computer.

Manual users will often have to stop the IconNMR run, close the TopSpin software, and logout the Walkup user before logging in themselves and running. **Do not run manual experiments in the Walkup Linux account**. The staff, on behalf of the other users, also requests that manual users note in their reservation comments that their plan on taking the system out of IconNMR and tell the staff about the plan. Sometimes the staff will ask a manual user to return the system to running IconNMR in the Walkup account, especially if the manual run takes place after hours.

Loading Samples

Insert your sample into a spinner and adjust its height using the square depth gauge. **This is extremely important**. For samples with limited solvent, center the solvent on the smallest black coil indicator or the probe center line on the side of the tool. We do have a few 3 mm spinners, but you must make arrangements with the staff to borrow one and it must be returned to a staff member promptly. Clean the tube and the spinner housing with a Kimwipe before placing them in the NMR instrument. **Do not spin the sample tube during your experiments**, as this is not necessary and can cause problems. Place the sample in the autosampler unit on the magnet, be sure it is not overheight, and note the sample position.

When running the system in manual mode the command to change the sample is: sx <sample position> (sample exchange). For example, if you placed the sample in position 7, “sx 7” will remove the current sample and place yours in the magnet.

This system should always have a sample in the magnet. When idle the sample in position 24, which is the 90:10::H_2O:D_2O sample, should be left in the magnet. This system is constantly working to improve the shims, which requires a locked sample. Therefore, when your experiments are done, please make sure that the water sample is back in the magnet, and that it has been locked, shimmed, and auto-shimming is on.

Collecting NMR Data

Setting Up a New File

Unlike when running in IconNMR, when running in manual mode you may overwrite data, so be careful not to do so accidentally. To start a data set type new or under the File (disk stack) menu choose New. A window will appear, which has four sections: Dataset, Parameters, Advanced, and Title.

In the Dataset section you are providing the information for the file structure. Bruker data is stored in your data Directory (usually /data/<login>) in a NAME directory, under which are directories of EXPNO numbers, under which are directories of PROCNO numbers. The NAME is often a project or sample identifier, and should not contain non-alphanumeric characters other than dashes and underscores. Each EXPNO number designates a separate experiment, which is often a separate acquisition (for example, a proton in 10 and a carbon in 11). PROCNO numbers are either where the experiment is repeated (like an optimization), processed with selections (like 2D phasing), or otherwise manipulated (like adding another experiment to the current one). In this section, you need to check that the NAME, EXPNO, and Directory are correct. Whether you choose (with the checkbox)

to open the new dataset in a new window or not is a matter of convenience.

In the Parameters section you need to check the radio button for the parameters to be used in the new experiment. Usually, you will choose “Read parameter set” and then click the “Select” button (see *Retrieving Parameters* below). However, sometimes the parameters from a previous experiment may be preferable, as they might already have adjustments you want to duplicate (like offset or spectral width). In this latter case, have the experiment from which you wish to use the parameters open and choose the “Use current parameters” radio button. Then you may check the “Set solvent” box and choose your solvent here, or this will be done for you when you lock the sample. You may also have it perform additional actions; but, usually, “Do nothing” is chosen.

Leave the Advanced section alone.

Finally, you may fill out the Title section here, or you may write it after the dataset has been created.

Retrieving Parameters

This process loads default parameters necessary to run your experiment. Usually, this step is done as part of Setting Up a New File (see above), but the command rpar also opens a window listing parameters for given experiments. There are two levels of parameter setup files: system and user. In general, you should use the user level of parameters, which may be accessed by adjusting the dropdown menu in the upper right to the ../user directory. Regardless of which parameter set you choose, **remember that this cryoprobe is limited to acquiring ^{1}H and ^{13}C data and only decouple ^{15}N and ^{2}H**. Some experiments of interest are:

PROTON_TAMU	^{1}H spectrum
CARBON_TAMU	^{13}C hydrogen inverse-gated decoupled spectrum
dept90hw	^{13}C hydrogen decoupled spectrum showing only CH peaks
dept135hw	^{13}C hydrogen decoupled spectrum with CH and CH_3 peaks positive, CH_2 peaks are negative
WATERGATE_TAMU	^{1}H WATERGATE solvent suppression
COSY_TAMU	^{1}H-^{1}H phased COSY for organic solvents
TOCSY_TAMU	^{1}H-^{1}H TOCSY mixing time 200 ms
NOESY_TAMU	2D NOE through-space correlation
HSQC_TAMU	^{13}C-^{1}H correlation with carbon decoupling during data acquisition
HMBC_TAMU	^{13}C-^{1}H long range correlation with single bond suppression

It is possible to obtain acceptable spectra for samples dissolved in 90% H2O-10% D2O by suppressing the water peak. The center of the spectrum (O1) must be the center of the water peak, 4.7 ppm at 305 K. There are some additional parameters sets to facilitate data requiring solvent suppression:

WATERGATE_TAMU	H_2O suppressed 1D hydrogen spectrum

1Hprhw	H_2O suppressed 1D hydrogen spectrum
COSY_WaterSat_TAMU	H_2O suppressed COSY
NOESY_WaterSat_TAMU	H_2O suppressed nOe 2D
ROESY_PRESAT_TAMU	H_2O suppressed ROESY
TOCSY_PRESAT_TAMU	H_2O suppressed TOCSY

Do not use any other parameter files without asking first, as they probably would not work properly and have the potential to damage the instrument.

Temperature

Temperature stability is important for shim stability and peak resolution. The instrument can control temperature in the range between 1 ℃ and 60 ℃. Temperature is natively reported in degrees Kelvin, and should be left at 305 K. To change the temperature, type edte and select the temperature button in the window that opens. Set the new temperature and then accept the value. It can be adjusted between 274 K and 343 K (1-70 ℃), but **you are not to underceed 283 K (10 ℃) or exceed 313 K (40 ℃)**. Always consider the melting and boiling points of your solvent when selecting the temperature. You may minimize or close the edte window when temperature is stable.

Locking and Shimming

Locking your sample is almost not an option. Yes, you may run a sample unlocked in manual mode, but it should be rare that you do so. To lock and shim your sample do the following:

1. Wait until the temperature is stable.
2. Type lock or click on the Lock button under the Acquire tab, then pick the most appropriate solvent with the cursor, click on it and click the button in the lower left to accept the value, then observe the lock process.
3. The lock signal should stabilize above halfway up the lock window. If the lock fails you probably have a sample issue, reload the 90:10::H_2O:D_2O sample and check if it locks. If the lock fails on the water sample: contact the NMR staff. Otherwise, it's your sample.
4. Automatic shimming requires a lock, and can be set up by typing: topshim gui, which opens the control window.
    a. Shim dimension should be 1D, optimized for the current solvent and proton, and set Tune before and Tune after both to Z.
    b. Press the Start button at the bottom of the window and wait until a "Topshim completed" message appears in the lower left below the text window.
    c. After shimming the Topshim window can then be minimized or closed using the buttons at top right. It is also possible to reach the Topshim gui by using the Shim button in the Acquire menu, then going to Additional Topshim Options and clicking on Topshim gui.
5. If the sample is dissolved in 90% H_2O-10% D_2O, the 3D version of shimming can be used. It takes more time but sometimes allows for better water suppression.
6. Often shimming on this system is done with the convection compensation (convcomp) option.
7. When the automatic shimming fails, this is almost always a sample issue. Reload the 90:10::H_2O:D_2O

sample, then lock and shim it. If the shimming fails on the water sample, reload the most recent set of 3D shims, then try to lock and shim the sample again. If the shimming still fails, contact the NMR staff.

8. The system can continue shimming a sample on which it has a lock during idle time. To enable this feature enter the command “autoshim on”, or start automatic shimming in the BSMS.

Tuning and Matching

Each NMR sample must be tuned and matched for every nucleus being pulsed, failure to do so can damage the probe. The command atma (or clicking on the tune button) performs automatic tuning and matching for the nuclei in the experiment you have presently in the foreground. Wait until the tuning and matching are finished for every nucleus before proceeding, the system will show an “atma finished” message at this point. The temperature should be stable, and you should tune and match every sample. If there is any doubt that the system has been fully tuned and matched for a given experiment, re-run the process with the experiment in the foreground.

If the tuning and matching fails, you likely have a sample issue. The most common problem that leads to samples not tuning and matching is the ion concentration. The higher electrical conductivity of salty solutions reduces the penetration depth of the RF, spoils the Q factor of the resonance circuit, and introduces additional sample noise. Cold probes are especially susceptible to salt, so **5 mm sample’s total ionic concentrations must be kept under 250 mM**. If circumstances cannot allow a sample to meet this criterion, then either consider a 3 mm tube (which will allow about twice as much salt) or do not attempt to run the sample on the cold probe.

Regardless of why, should the tuning and matching fail: contact the staff immediately. Do not under any circumstances continue with your experiments. Put a note on the keyboard that the system is down, and contact the staff. Delivering even a single RF pulse on an un-tuned cold probe could damage the probe.

Acquisition Parameters

User level parameter files come with a set of values for their designated experiment, but there is a chance that those values may have changed since the parameter file was created. Click (or type) getprosol to tell the software to reconcile the current experiment’s setup with the values currently in the probe file. System level parameter files are often completely empty and depend on this import of values.

Click on the ACQUPARS page above the data window or type eda or ased. Check the number of scans, sweep width, pulse lengths, and other adjustable features. If you are familiar with Bruker pulse program coding, check the PULSEPROG page if you are unsure what additional parameters may need adjusted. When adjusting parameters keep the system’s limitations in mind; for example, **do not increase the ^{1}H sweep widths or the power settings for decoupling in the HSQC or HMBC experiments** as such changes may put too much power into the broadband channel, which can damage or destroy the instrument.

There are many parameters, but those most commonly adjusted are:

ns Number of scans - This is the number of iterations of the pulse sequence that will be added together to generate the final spectrum.

d1 Relaxation delay - This is the delay (in seconds) between each scan that allows the magnetization to reach an acceptable (pseudo) equilibrium.

aq Acquisition time - This is the time (in seconds) that the signal is recorded for each scan.

sw Spectral width (in ppm) - This is how wide a frequency the spectrum covers.

o1p Transmitter frequency offset (in ppm) - This is the center to the acquired spectrum.

Choosing the appropriate number of scans is a matter of budgeting your reservation time and judging other results from the sample. For example, if you acquire a proton on the sample first, you can often judge how to run other experiments on that same sample - there are aids available that demonstrate this sort of decision. The choice of relaxation delay is often one governed by the sample and the goal of the acquisition. For example, if you really want to see the carbonyls in a sample you are going to need a lengthy relaxation delay. The acquisition time should be set long enough to capture the whole signal of interest, but not much longer. It is also important to consider the decoupler during acquisition, especially for broadband decoupling. The spectral width and offset should be set together to place the whole spectrum comfortably within the spectrum. Furthermore, remember that adjustments to the spectral width will change the acquisition time. Finally, if the experiment requires water suppression, the o1p value must be set on the water peak (~4.7ppm).

You are expected to complete your experiment before the end of your reservation. Once you have chosen your parameters, be sure to check the time (by typing expt or clicking the clock icon) required to run the experiment.

Receiver Gain

Type rga or click on the left side of the Gain button in the Acquire tab to set the receiver gain for best sensitivity without data overflow. In other words, when your data is digitized you want it to be close to using the available bits without going over, which provides your data the best digital resolution. In case 2D experiments often it is better/necessary to use the gain from a previous 1D with the same detected nucleus. Setting the gain manually can be performed by typing rg <#> or by clicking on the right side of the Gain button and setting the value in the pop-up window.

Collecting Data

1. Use the command expt (or a click on the clock above the data window) to calculate the total experimental time using your setup data. You must be done with your experiment by the end of your reservation.
2. Type zg or click on the green arrow to start the experiment.
3. During a 1D experiment, the data may be viewed by first transferring the data to the computer by typing tr (or pulling down the arrow to the right of the Green Go Arrow and selecting the data transfer option) and then typing efp. For 2D experiments the transfer is not necessary, once enough t_1 points have been acquired the current spectrum may be displayed by typing xfb. Clicking the left portion of the Proc. Spectrum button under the Process tab performs these steps automatically.
4. To stop an experiment type halt (or click on the square red button), and the system will finish its current step, save the data, and go idle. If you use the stop button the system immediately ceases its work, does <u>not</u> save the data, and goes idle.

Data Manipulation

Buttons above the data window control location, intensity, movement, et cetera and can be confusing. Mousing over a button brings up a small pop-up that explains its function. Fortunately, unlike instrument control functions, little damage can be done with these functions. This is a good point to mention that **TopSpin is free to academic users**, by registering for an account with Bruker **using your tamu email address** and you may download and install a copy of the software for processing on your own computer. Furthermore, the NMR facility's workstations (proton and deuterium), which already house your data, have TopSpin on them, so feel free to make use of them

and ask questions. Finally, the NMR staff has some helpful videos for some commonly asked TopSpin questions.

1D

The basic data viewing functions are controlled by a group of buttons located directly above the data window.

Doubling or halving the spectrum amplitude can be controlled by the *2 and /2 buttons. The two vertical triangle button is a click-and-drag amplitude adjustment, and the two horizontal triangle button is a click-and-drag frequency zoom. The opposing vertical arrows are a click-and-drag vertical offset, the two horizontal opposing arrows are a click-and-drag frequency scroll, and the arrow pointing to a line is a tool to place the baseline near the bottom of the window. The two magnifying glasses are set zoom functions, and the left swinging arrow is an undo for them. However, usually, when expanding a section of the spectrum it is easier to move the cursor over the spectrum to one side of the area to be expanded, left click and pull the cursor to the other side of the area and then release the mouse button, which fills the data window with that section. The box with four opposing arrows is a full reset, showing the entire spectrum in the full data window. The circling arrows and bar/box buttons that follow are for synchronizing and region preservation between datasets, respectively. The two angles with a line above them button is for measuring frequency differences on the spectrum using the mouse, by clicking-and-dragging on the spectrum. The button with two mock spectra is for setting up and displaying multiple spectra at the same time. And the button with two chevrons expands the tool (which includes an exact zoom tool, which is especially useful before plotting).

Useful commands for 1D data are apk for automatic phasing and abs for automatic baseline correction. Clicking the left portion of the Proc. Spectrum button actually performs several functions including line broadening and automatic phasing, but sometimes these need to be further adjusted by hand. Buttons under the Analyze tab allow you to pick peaks, integrate, et cetera.

One further word, there is a PROCPARS page with your data. It governs how the spectrum is initially processed, and may need to be adjusted, especially if you changed your acquisition time.

2D

For 2D data the basic processing bar looks similar, but there are a few changes.

The *X button changes the spacing between the contours, the opposed triangles pointing to lines button resets the contours to the spacing last saved, and the disk with the contoured peak button saves off the current contour spacing. The four opposed arrows is a drag-and-pull button that shifts the spectrum around in the window. Usually for 2D data zooming in on a region is done by left clicking in the spectrum and then pulling across the area to be expanded when the left mouse button is released. However, the same zoom control tools from the 1D bar are present, including the four opposing boxed arrow button that returns the entire spectrum to the full display. The measuring tool does work differently in 2D work, in that the measurement is taken diagonally across a rectangle you create by clicking-and-dragging.

Projections are initially produced by adding intensity of all spectral points in the 2D spectrum, but you may replace

them with higher-quality 1D data, if it is available. To replace the 2D projection: right click on the projection area, choose the External Projection option and then enter the file specifications of the 1D spectrum. The square button at one corner of the projection area allows you to use the basic processing functions to adjust the projection. Remember to toggle the projection area square to return to 2D manipulations.

Printing and Plotting

This is not as easy as it seems… First, and foremost, if you are happy with how the spectrum area looks and you want a hardcopy of it: just go to the File (disk stack) button and Print it. If you want to create a template with a parameter sidebar, charming logo, boxes and arrows, et cetera: go to the Plot tab above your data and get all that set up, then go to the File (disk stack) button and Plot it. Do not try to plot your template from the Plot tab - it just does not work. Setting up the plotter template is a great way to produce exactly the same picture across multiple datasets.

Data Export

If you would like to export the data window to a graphics file choose Export from the File (data stack) button. A window will pop-up with a Save As field, where you give it a file name with the extension of the appropriate type of file. Supported formats are .png, .jpg, .jpeg, .bmp, .wmf, and .pdf. If you'd like the data as a text file type convbin2asc on the command line with the data open in the foreground, and an ascii-spec file will be generated in the data's pdata/<procno> directory with columns Point #, Intensity, Frequency (Hz), and Chemical Shift (ppm), respectively.

Finishing

When you are finished acquiring your data there are a few things you need to do, which should still fit within the timeframe of your reservation.

1. Return the system to 305 K and a gas flow of 535 L/hr, and allow the system to stabilize.
2. Run the sw24 macro and allow it to complete. This macro removes your sample, inserts the 90:10::H_2O:D_2O sample in position 24, and then shims the new sample. **Wait for this macro to complete**.
3. Close the TopSpin software.
4. Logout of your Linux account. Do not choose "switch user", and do not power down the computer.
5. Inform the staff that the system needs to be returned to running IconNMR. (It would be very nice of you to do this ahead of time. In fact, please put in your reservation comments that you will be running the system outside of IconNMR. We may provide instructions for you to return the system to running IconNMR, especially if your reservation is after hours).

Special Topics

Running Multiple Acquisitions

The best way to run multiple acquisitions, especially across different samples, is to use IconNMR. However, if you are working with an application that cannot be done in IconNMR, set up each experiment with unit changes in experiment number in order within the same Name. Then do all of the initial set up steps (load, lock, tune and match (all relevant channels), and shim the sample). Then load the first of these experiments in the foreground and type multizg, and tell it how many experiments to run. If you tell TopSpin to run more experiments than you set up, it will repeat the last of those experiments you did set up until it reaches the number with which you

answered. It is possible to adjust parameters in experiments which have not been started without harming those which are in progress, but the system will not report the change in acquisition time for these changes.

The multizg method of acquisition is only useful on a single sample. And there must not be any reason to re-tune the system (so no temperature changes, et cetera).

Kinetics

NMR is unique in its ability to observe intermediates in a chemical process and also to observe the rates of change without any manipulation of the sample. To perform a kinetics study, first determine the time and factors (temperature, substrate to reactant, catalyst or enzyme concentration, et cetera) necessary to make the total change. Then, using the starting material, determine the concentrations necessary to collect spectra with usable signal/noise on the sample. Next you need to decide if you can run the experiment at all, determine the total time required, and book the system for that time.

On the next visit, start with an initial spectrum with the substrate. Type new, move to experiment 2 with current parameters. Remove the sample from the magnet, add the other reactant(s)/catalyst/enzyme, mix or vortex the sample and then return to the magnet. Make sure the sample loads, locks, tunes and shims. Then type multizg and when asked how many experiments, divide the total experiment time by the time for each spectral accumulation (say 2 hours of 5 minute experiments, 2x60/5=24) and enter that number or less. Do not enter a larger number as halting a multizg can be difficult, and you should not exceed your reservation time. After transformation, phasing and so on, the command stack1d may be used to display and print the results.

T_1 Measurements

T_1 is the longitudinal (or spin-lattice) relaxation time, and is the decay constant for the recovery of the z component of the nuclear spin magnetization to equilibrium. The system has done these experiments well (for both ^{1}H and ^{13}C), and they may be run in IconNMR. Please refer to our Bruker T_1 Measurements document for details.

DOSY

Diffusion Ordered SpectrscopY is a method of using the NMR to observe the (vertical) translational motion of targets in a sample. Please refer to Bruker's DOSY and Diffusion by NMR manual for details on the technique. To be done well, a DOSY requires significant setup and calibration. Please check with the NMR staff for additional help and instructions before attempting this technique, but the system has proven quite capable in performing it.

Temperature Work

The system is capable of running experiments that follow a list of temperatures; however, you must remember the limitations of the cold probe. Please check with the NMR staff for additional help and instructions before attempting this procedure. Additionally, temperature standards are available, should you need them.

Appendix

Bruker Pulse and Power Recommendations

All values require a minimum *d1* + *aq* of 1s. Do not exceed the indicated limits!

	5 mm 500 MHz TXI/TCI Cryoprobe Values
^{1}H	
hard pulse (max length 360°)	8.0 μs
hard pulse for lossy samples	Power level corresponding to 8.0 μs pulse for non-lossy sample
trim puse p28	2 ms @ 10 μs
TOCSY spin lock	Up to CW for power level corresponding to a 100 μs pulse
ROSEY spin lock	Up to CW for power level corresponding to a 100 μs pulse
DIPSI2 decoupling in triple resonance	400 ms @ 35 μs
^{13}C	
hard pulse (max length 360°)	12.0 μs
trim pulse	2 ms @ 25 μs
CC spin lock	20 ms @ 25 μs
GARP4 decoupling	140 ms @ 65 μs
selective pulses	Q5: 384 μs Q3: 307 μs CHIRP: 2 ms @ 25 μs
^{15}N	
hard pulse (max length 360°)	35.0 μs
GARP4 decoupling	140 ms @ 200 μs
CPMG T2	250 ms @ 63 μs
^{2}H	
hard pulse (max length 360°)	68 μs
WALTZ64 decoupling	100 ms @ 250 μs
Z-Gradient	
Absolute maximum current	10 A
Maximum overall length	10 ms @ 10 A

System Performance

These are values typically observed on our system. Do not exceed the indicated limits!

	Non-lossy t90 (μs)	Power Limit (dB)	Power Limit (W)	Time Limit
^{1}H				
Amplifier: A2	8	8	3.874	32 μs
SGU 2	10	9.94	2.479	2 ms
	25	17.9	0.397	200 ms
	35	20.82	0.202	400 ms
	100	29.94	0.025	CW
^{13}C				
Amplifier: A1	12	0	75.608	48 μs
SGU 1	25	6.38	17.420	20 ms
	65	14.67	2.577	140 ms
^{15}N				
Amplifier: A3	35	1	79.810	140 μs
SGU 3	200	16.14	2.444	140 ms
^{2}H				
Amplifier: A4	68	0	31.999	272 μs
SGU 4	250	11.31	2.323	100 ms