

Experiments in ICONNMR (Available on the Open Access NMR Instruments)

Submitting your Sample to the NMR spectrometer

After preparing your NMR sample, you should perform the following steps in order to acquire an NMR spectrum from either one of the Open Access NMR instruments (Nano400 or DPX400).

- Inspect the display on the computer which represents the activity of the 60-position samples in the BACS sample changer. Assuming that other users of the NMR system have obeyed the procedures for sample submission which are described in this section, then holders which are coloured green will be empty; holders which are coloured yellow will contain samples which have been run, but have not yet been removed from the carousel; samples which are coloured pink are waiting to be run in the NMR “queue”; and the sample which is coloured red is currently being acquired. Make a note of the number of the next available sample position (coloured green) at the end of the “queue” of pink samples.
- Make sure that some form of identification has been written in felt tip pen at the top of tube (no sticky labels, please!). NMR samples which are not collected from the carousel on the day that they were submitted will be removed at the start of the next day. If you fail to identify your sample in this way, and you do not remove it from the carousel on the day when it was submitted, then you may not be able to find your sample amongst all the others which have been removed.
- Clean the outside of the NMR tube with the tissue paper provided before inserting it into a blue spinner turbine (any residual grease on the surface of the NMR tube will adversely affect the performance of the instrument).
- Set the correct depth for the NMR tube in the spinner using the sample depth gauge provided.
- Place your sample on the carousel in the first available slot after the other “queued” samples (which corresponds to the first “green” sample at the end of the row of “pink” samples on the computer’s representation of the BACS sample changer). Daytime samples are run in numerical order, so following this procedure ensures that your sample will be run as quickly as possible. (However, note that night time samples are run in order of submission).
- Click the “Change User” button in the ICON NMR display on the computer in order to begin setting up experiments on the sample which you have just loaded on the carousel. Select your individual account name and enter your password (both of which will have been assigned to you at the session for Training in Use of the Open Access NMR Instruments). By logging onto your own account at the start of a session you ensure that your data is saved in your personal data storage area - making it easy to find archived data again at a later date. The individual password for

your account ensures that only you are able to save spectra in this data storage area, so you should not give your password to anyone else!! (Also, if someone else damages the instrument while logged in under your account you may be blamed...both of the 400 MHz instruments maintain a log of user activity on the system).

- In the main ICONNMR display, double click on the sample holder number which contains your sample. Chose a solvent (the default is CDCl_3), then chose an NMR experiment (the default is a 1D- proton experiment – but see below for a list of other possibilities) and then enter a title which will describe your NMR spectrum. The choice of title is up to you, but it is suggested that, at a minimum, the title should incorporate your initials (so that you can quickly identify your spectrum amongst all the other printouts from the NMR spectrometers) and a reference to your laboratory notebook (so that you can find the spectrum amongst all the others which will be generated during your research). After checking that all these details are correct, click the “submit” button to add your sample to the NMR queue. If all is well, then your sample should turn from green to pink on the representation of the BACS sample changer. Finally, click on the “Change User” button again to log out of your account (note that both Open Access NMR instruments will automatically log out of your account after 2 minutes of keyboard inactivity).
- Now that your sample has been submitted to the NMR queue, take a glance at the bottom of the main ICON NMR screen. For daytime samples, this will tell you how long it will be until your sample (the last in the queue) has been run.
- When you return to the CAF lab after this calculated amount of time, your sample should have changed from pink to yellow. Collect your spectrum (printed out in black and white on A4 paper) from the printer and remember to **also collect your sample from the carousel and delete its entry on the main ICONNMR screen** (the entry on the representation of the BACS sample changer will change from yellow to green when you do this). Samples which have not been collected on the day when they were submitted will be removed from the carousel on the next day. They will then be kept for one week, after which they will be disposed of.

If you simply want to record a ^1H NMR spectrum, then please submit your sample to whichever instrument has the shorter “queue”. For other experiments, however, it is worth considering the relative sensitivities of the Nano400 and DPX400, as set out in the Sample Requirements for Experiments in ICONNMR. In general terms:

- The Nano 400 has better sensitivity for 2D-NMR experiments
- The DPX 400 has better sensitivity for ^{13}C and DEPT NMR experiments

By default, the following ICONNNMR experiments are available on the two Open Access NMR instruments:

Experiment	Nano400 (Acquisition Time: mins:seconds)	DPX400 (Acquisition Time: mins:seconds)
¹ H	N Pro4ppm.ph "Proton 4 Scans" (00:36)	N Pro8ppm.ph (0:55)
¹ H wide sweep width	N Pro4acid.ph "Plot includes acid peaks" (00:36)	-
¹ H water suppression	N WATERSUPPRESSION.ph (1:36)	N WATERSUPPRESSION.ph (2:29)
¹³ C	N Carb128.ph "C13 ns=128" (6:12)	N Carb128.ph "ns=128" (6:31)
¹³ C longer acquisition	-	N Carb 640.ph "ns=640 31 minutes" (31:31)
¹³ C very long acquisition	-	N Carb 1280.ph "ns=1280 1hr" (1:02:46)
DEPT-135	N DEPT135.rdg "CH ₃ and CH positive; CH ₂ negative; quaternary absent" (3:51)	N DEPT135.ph "ns=64 3.5 mins" (3:56)
DEPT-135 longer acquisition	-	N DEPT135_long.ph "ns 320 18 minutes" (18:29)
DEPTQ	N DEPTQ.ph "like DEPT 135 but with Quaternary carbons" (4:05)	N DEPTQ.ph "DEPT135+Quaternary carbons" (ca 4 mins)
Edited-HSQC	C HSQCedgr.ph "sweep optimized HSQC with gradients" (7:43)	C HSQCedgr.ph "Sw opt. Edited HSQC with gradients (e/a TPPI)" (5:18)
HMBC	C HMBC.ph "Sweep Optimized HMBC with gradients" (8:31)	C HMBC.rdg (4:47)
DQF COSY	C COSY.ph (3:53)	COSY.ph "Sw. Opt. COSY" (3:58)
NOESY	C NOESY.ph (45:40)	-
The Lot (¹ H, COSY, DEPT135, DEPTQ, HSQC, HMBC)	C The Lot (0:36+3:53+3:51+4:05+7:43+8:31)	C the Lot "{}" (0:55+3:58+6:31+3:56+5:18+4:30)

You should have first obtained a 1D- ¹H NMR spectrum from your sample before acquiring any of the heteronuclear 1D- NMR experiments (such as ¹³C and DEPT-135), or before proceeding to any of the 2D-NMR experiments (such as edited-HSQC, HMBC, COSY and NOESY). Running a ¹H NMR spectrum enables you to quickly answer two key questions regarding your sample, which will determine whether or not it is justified to proceed with such further analysis.

- Is there as much compound in the NMR sample as you expected? (see Sample Requirements for Experiments in ICONNNMR)
- Is the sample sufficiently pure that you are likely to obtain meaningful results from running further NMR experiments?

If the ¹H NMR spectrum shows that your sample fails on either of the above criteria, then there is no point in proceeding to collect further NMR data. Not only will you be wasting your own time, but you will potentially be preventing other users of the Open Access NMR instruments from running their spectra. The NMR Staff constantly monitor the use of the Open Access Instruments and reserve the right to delete the accounts of users who persist in running samples which are simply a "waste of time", according to the above criteria.

Most of the above NMR experiments require roughly 1-7 minutes of acquisition time (between 5-12 mins of spectrometer time) and there is no restriction as to when each experiment may be run on the Open Access Instruments. Such experiments are designated as “day time” - a “sun” icon will appear against them, when submitted in ICONNMR. There are a few exceptions to this, however, such as long carbons and the long DEPT-135 on the DPX400 and the composite experiment “the Lot” (^1H , ^{13}C , DEPT-135, edited HSQC, HMBC and COSY), which is available on both Nano400 and DPX400. All of these experiments require a significantly longer period of time for acquisition, and are accordingly designated as “night time”. An icon for a crescent moon appears against these experiments when they are submitted to ICONNMR. It is possible to submit “night time” experiments to ICONNMR at any time, and such experiments will appear pink in the representation of the BACS sample changer, as normal. However, “night time” experiments will not be added to the “queue” of day time NMR experiments. Rather, such experiments will be acquired between the hours of 18:30 and 9:30 (next day) on the DPX 400 and between 21:00 and 9:30 (next day) on the Nano400, in an order which is determined by the time at which they were submitted (i.e. the order for “night time” experiments is NOT dependent on numerical position on the carousel, as was the case for the “day time” experiments).

It is possible to change the designation of an experiment from “day time” to “night time”, simply by clicking on the “sun” icon during the submission process. This changes the icon from a “sun” to a “moon”. If the Open Access Instruments are very busy, and if the NMR spectra from your “day time” ^{13}C , DEPT-135, edited HSQC, HMBC or COSY experiments are not required immediately, then please consider designating these experiments as “night time”, rather than “day time”, as a courtesy to other users who may have more urgent samples.

Sample Requirements for Experiments in ICONNMR

By default, the two Open Access NMR instruments run more or less the same series of NMR experiments (^1H , ^{13}C , DEPT, HSQC, HMBC, COSY and NOESY). However the relative sensitivities of the two instruments differ significantly and this can be an important consideration when choosing which instrument to use, if you have a limited amount of sample. The minimum amount of sample which is required in order to obtain a reasonable NMR spectrum in the default length of time for each of these “day time” experiments is given below for both the Nano400 and the DPX400 NMR instruments:

Nano 400 Sensitivity*

	Day/Night	ICON NMR	Time (mins:seconds)	Limit for the minimum amount of sample which is required in order to obtain an acceptable NMR spectrum
^1H	Day time	N Pro4ppm.ph “Proton 4 Scans”	0:36	50 μg
^{13}C	Day time	N Carb128.ph “C13 ns=128”	6:12	5 mg
DEPT-135	Day time	N DEPT135.rdg “CH ₃ and CH positive; CH ₂ negative; quaternary absent”	3:51	2 mg
Edited HSQC	Day time	C HSQCedgr.ph “sweep optimized HSQC with gradients”	7:43	0.5 mg
HMBC	Day time	C HMBC.ph “Sweep Optimized HMBC with gradients”	8:31	1 mg
COSY	Day time	C COSY.ph	3:53	50 μg
NOESY	Night time	C NOESY.ph	45:40	2 mg
“The Lot”(^1H ; COSY; ^{13}C ; DEPT-135, HSQC, HMBC)	Night time	C The Lot	0:36+3:53+3:51+4:05+7:43+8:31	2 mg

*Results refer to artemisinin in 0.5ml CDCl_3

DPX400 sensitivity*

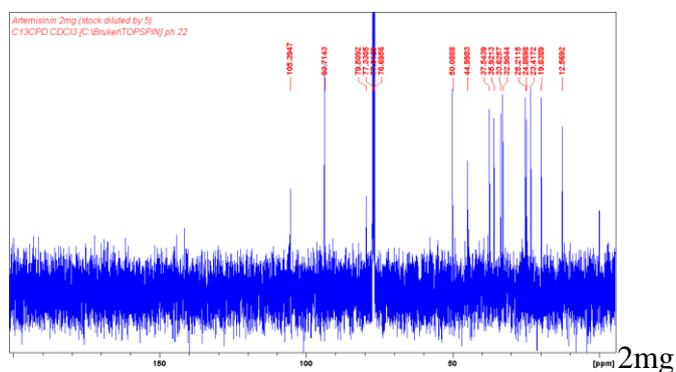
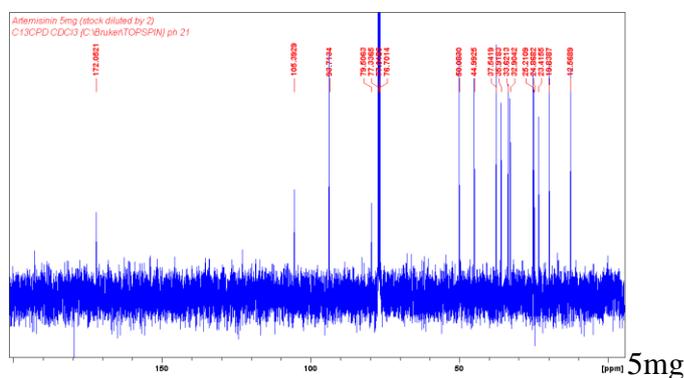
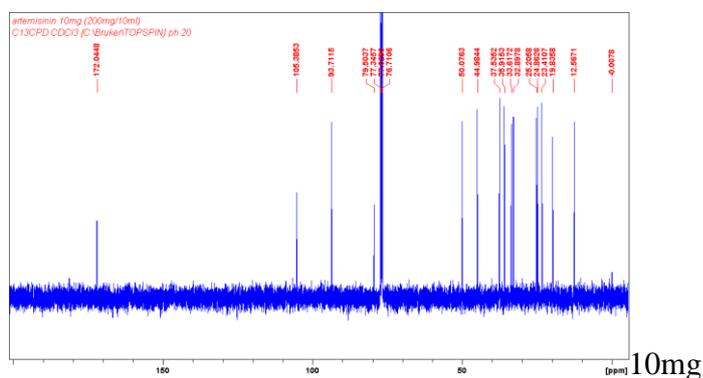
	Day/Night	ICON NMR	Time (mins:seconds)	Limit for the minimum amount of sample which is required in order to obtain an acceptable NMR spectrum
¹ H	Day time	N Pro8ppm.ph	0:55	0.2 mg
¹³ C	Day time	N Carb128.ph "ns=128"	6:31	2 mg
¹³ C long acquisition	Night time	N Carb 640.ph "ns=640 31 minutes"	31:31	ca. 1 mg
¹³ C very long acquisition	Night time	N Carb 1280.ph "ns=1280 1hr"	1:02:46	ca. 0.5 mg
DEPT-135	Day time	N DEPT135.ph "ns=64 3.5 mins"	3:56	1 mg
DEPT-135 long acquisition	Night time	N DEPT135_long.ph "ns 320 18 minutes"	18:29	ca. 0.5 mg
Edited HSQC	Day time	C HSQCedgr.ph "Sw opt. Edited HSQC with gradients (e/a TPPI)"	5:18	1 mg
HMBC	Day time	C HMBC.rdg	4:47	5 mg
COSY	Day time	COSY.ph "Sw. Opt. COSY"	3:58	0.2 mg
"The Lot" (¹ H; COSY; ¹³ C; DEPT-135, HSQC, HMBC)	Night time	C the Lot "{}	0:55+3:58+6:31+3:56+5:18+ 4:47	2 mg

*Results refer to artemisinin in 0.5ml CDCl₃

As can be seen from these Tables, the Nano 400 has better sensitivity for all 2D-NMR experiments, while the DPX 400 has better sensitivity for ¹³C and DEPT NMR. If you have a limited amount of compound therefore, it makes sense to submit your sample to whichever instrument is more appropriate, when running either 1D- or 2D-experiments. The effect of using less than the stipulated amount of sample with the daytime ¹³C, DEPT-135, edited HSQC and HMBC experiments are shown below, using the example compound, artemisinin, for the Nano400 instrument:

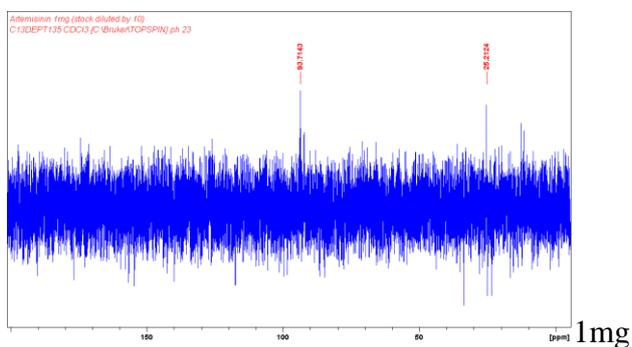
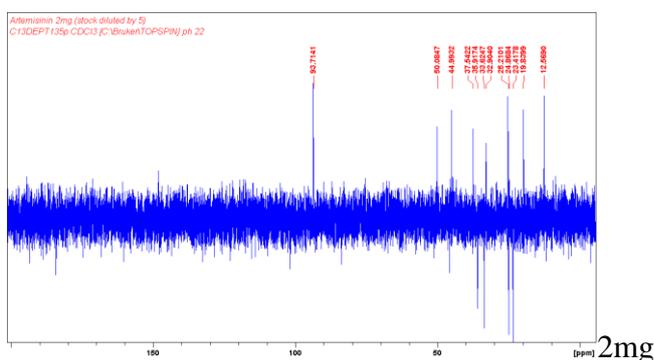
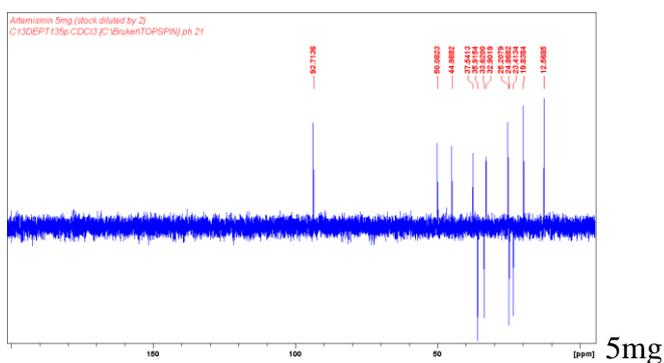
Carbon Sensitivity

Using the default day time Carbon-13 experiment, C13CPD, it is possible to see all 15 carbons of a 10 mg sample of artemisinin in just over 6 minutes (using 128 scans). Halving the amount of sample to 5 mg, gives results which are still acceptable, although the baseline noise is starting to become a problem. Reducing the amount of sample still further to 2 mg, results in a ^{13}C NMR spectrum which is unacceptable because a quaternary carbon at δ_{C} 172 ppm has now “disappeared” into the noise – if we did not know the structure of artemisinin we might wrongly assume that this compound incorporated only 14 carbon atoms! The solution to the problem for the 2 mg sample would be to consider using the DPX400 instead of the Nano400 and/or to choose a night time ^{13}C NMR experiment with a longer acquisition time. The relationship between sample size and acquisition time is shown in the two tables below, using artemisinin as an example, on the Nano400 and DPX400 instruments.



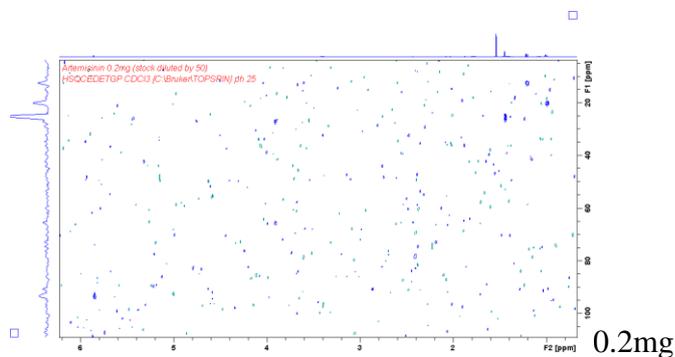
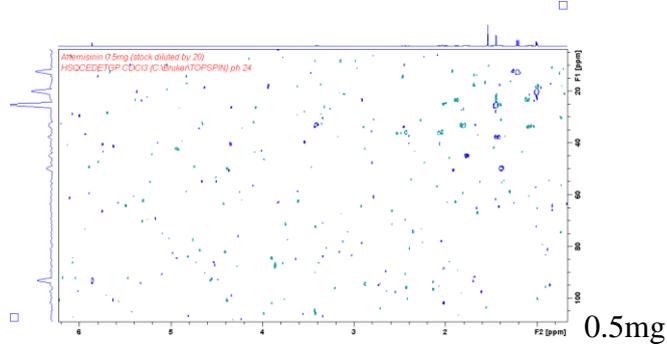
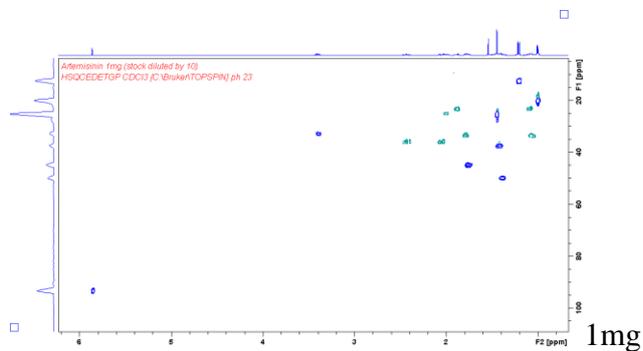
DEPT sensitivity

Using the default day time DEPT-135 experiment, DEPT135, it is possible to see all 12 methyl, methylene and methine carbons from a 5 mg sample of artemisinin in around three and a half minutes (using 64 scans). Reducing the amount of sample to 2 mg, gives a result which is just about acceptable, although the baseline noise is now comparable to many of the “real” peaks. Halving the amount of sample again to 1mg, results in a DEPT spectrum which is unacceptable because many of the peaks have now “disappeared” completely into the noise. The solution to the problem with the 1 mg sample would be to consider using the DPX400 instead of the Nano400 and/or to choose a night time DEPT experiment with a longer acquisition time. The relationship between sample size and acquisition time is shown in the two tables below, using artemisinin as an example, on the Nano400 and DPX400 instruments.



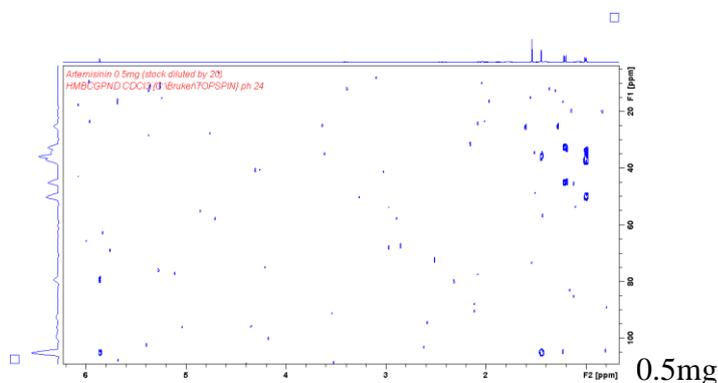
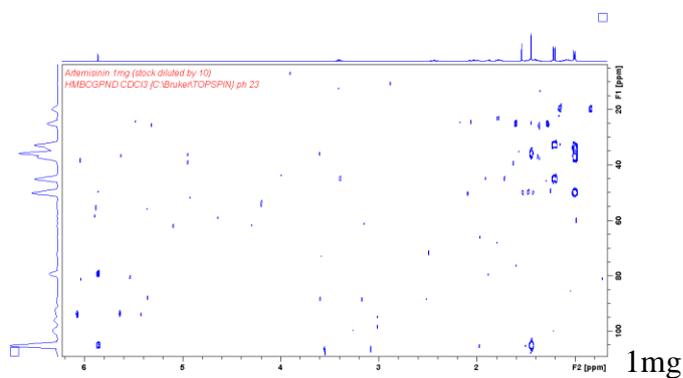
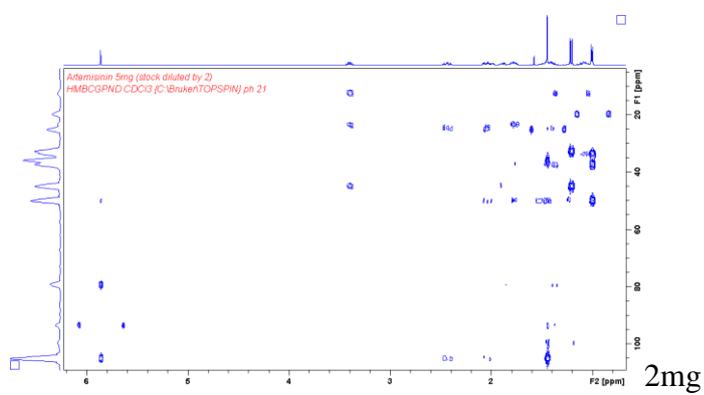
Edited-HSQC sensitivity

Using the default daytime edited-HSQC experiment, HSQCEDGR, it is possible to resolve two-dimensional resonances from all 12 methine, methylene and methyl carbons of a 1 mg sample of artemisinin in just over 5 minutes (using just 2 repetitions of the phase cycle). Halving the amount of sample to 0.5 mg gives results which are still acceptable, although the contour levels must now be increased to the extent that the baseline noise is unavoidably displayed alongside the real peaks. Reducing the amount of sample still further to 0.2 mg, results in a 2D-NMR spectrum which is unacceptable because most of the methine and methylene peaks have “disappeared” into the noise (although the more intense methyl peaks are still discernible). The solution to the problem with the 0.2 mg sample would be to increase the number of repetitions in the phase cycle from 2 to 4 (or even 8). The Nano400 always gives better results for this 2D-NMR experiment than the DPX400, as is shown in the two tables below, which illustrate the relationship between sample size and acquisition times, using artemisinin as an example, for the Nano400 and DPX400 instruments.



HMBC sensitivity

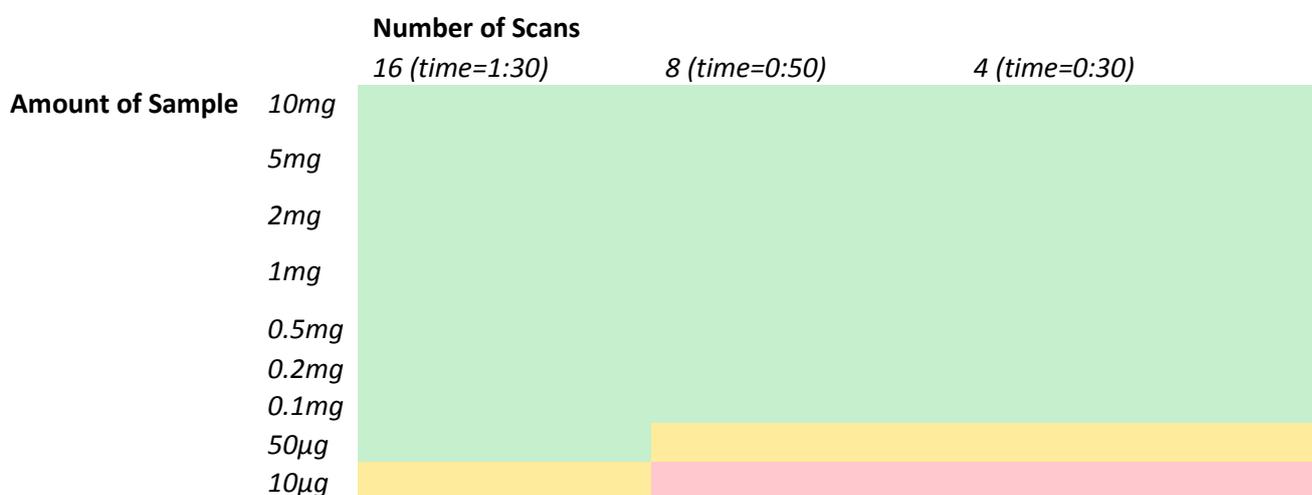
Using the default daytime HMBC experiment, HMBCGPND, it is possible to resolve many of the two- and three-bond connections for a 2 mg sample of artemisinin in less than 5 minutes (using just 1 repetition of the phase cycle). Halving the amount of sample to 1 mg, retains most of these correlations, with the penalty that contour levels must be increased to the extent that the baseline noise is now displayed alongside the real peaks. Reducing the amount of sample still further to 0.5 mg, result in a 2D-NMR spectrum in which many of the long-range correlations have disappeared into the noise, leaving only the 2- and 3-bond connections to the methyl groups (which are intrinsically more intense) still clearly discernible. The solution to the problem with the 0.5 mg sample would be to increase the number of repetitions in the phase cycle from 1 to 2 (or even 4). The Nano400 will always give better results for this 2D-NMR experiment than the DPX400, as is shown in the two tables below, which illustrate the relationship between sample size and acquisition times, using artemisinin as an example, for the Nano400 and DPX400 instruments.



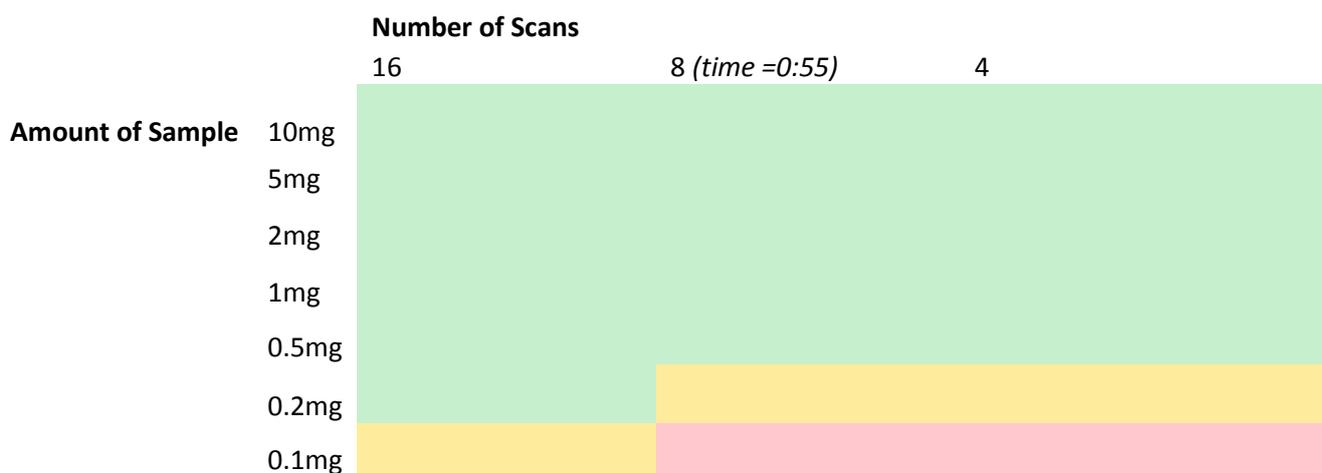
When a sample is present in only a limited amount, then it is possible to decrease the minimum amount of sample which is required in order to obtain a reasonable quality spectrum by extending the acquisition time (number of scans) for that experiment. As a general rule of thumb, increasing the experiment time/number of scans by a factor of 4 will decrease the minimum amount of sample which is required by a factor of 2. This interaction between time and sample requirement is shown graphically in the tables below for both the Nano400 and DPX400 instruments, using artemisinin as an example organic compound. Areas of the table which are coloured green correspond to “good” spectra; areas coloured yellow indicate spectra which are just acceptable; and red areas of the table correspond to unacceptable spectra.

PROTON SENSITIVITY

Nano400 (BBI) ^1H Sensitivity with artemisinin in 0.5ml CDCl_3 (ICON NMR - PROTON (zg30) NS=8; Time 0:50)

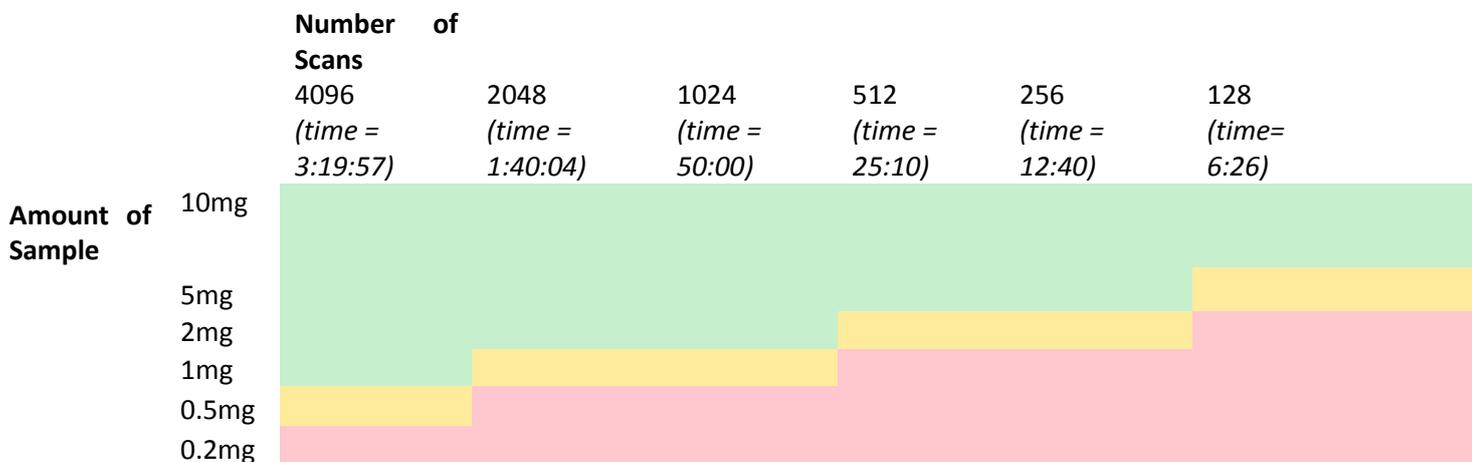


DPX400 BBFO ^1H Sensitivity with artemisinin in 0.5ml CDCl_3 (ICON NMR - PROTON (zg30) NS=8; Time 0:55)

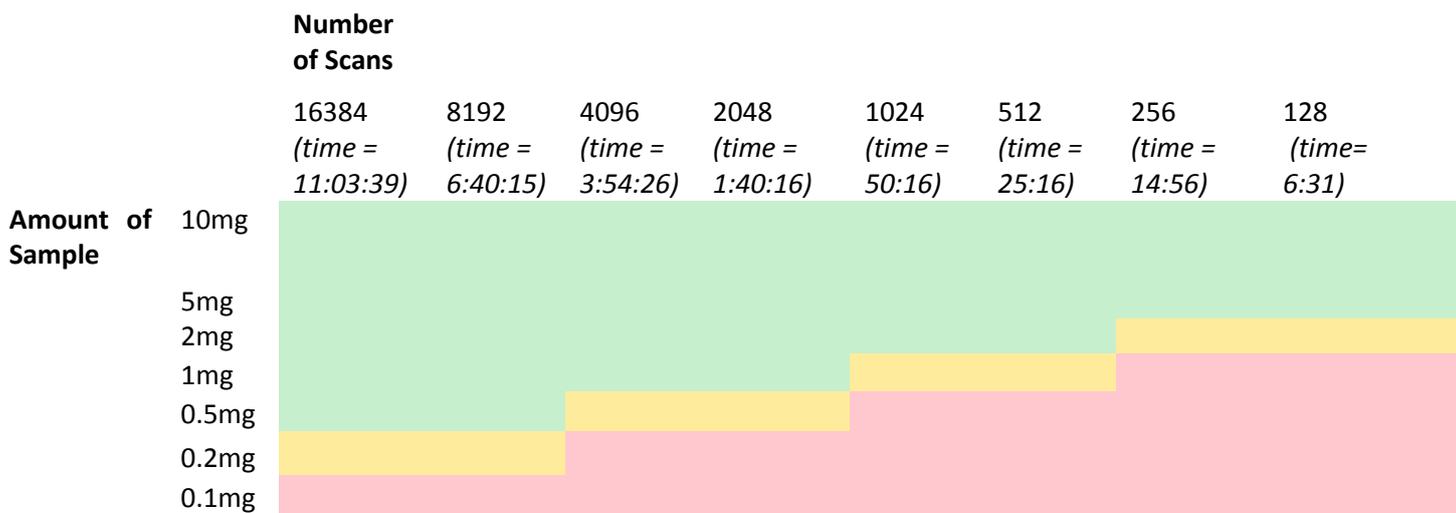


CARBON SENSITIVITY

Nano400 BBI ¹³C Sensitivity with artemisinin in 0.5ml CDCl₃ (ICON NMR - C13CPD (zpgg30)
NS=128; d1 =1.5; Time 6:26)

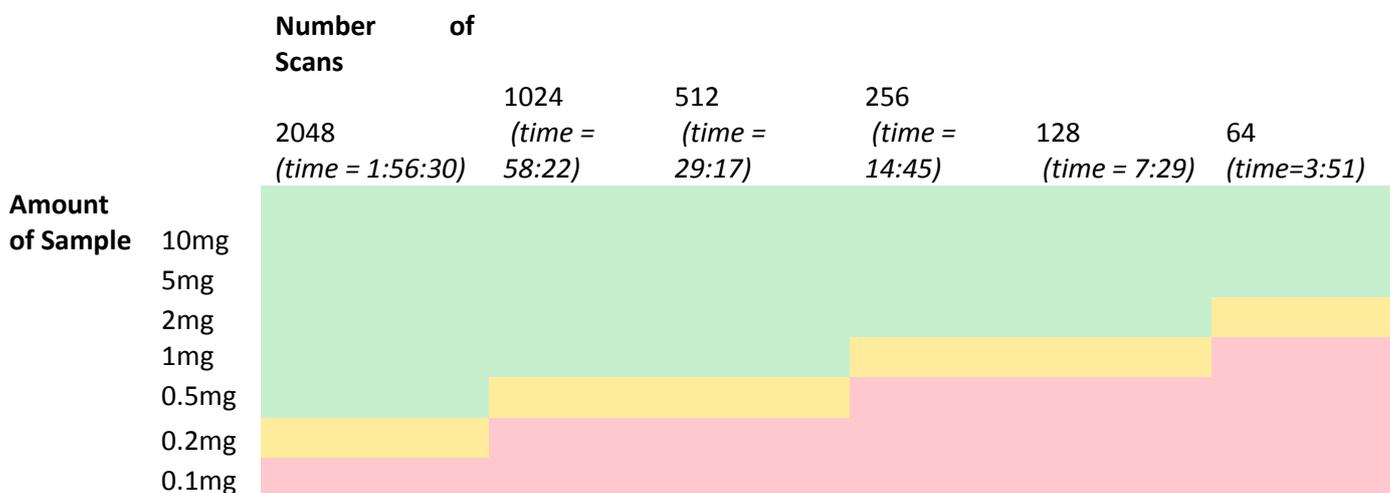


DPX400 BBFO ¹³C Sensitivity with artemisinin in 0.5ml CDCl₃ (ICON NMR - C13CPD (zpgg30) NS=128; d1 =1.5; Time 6:31)

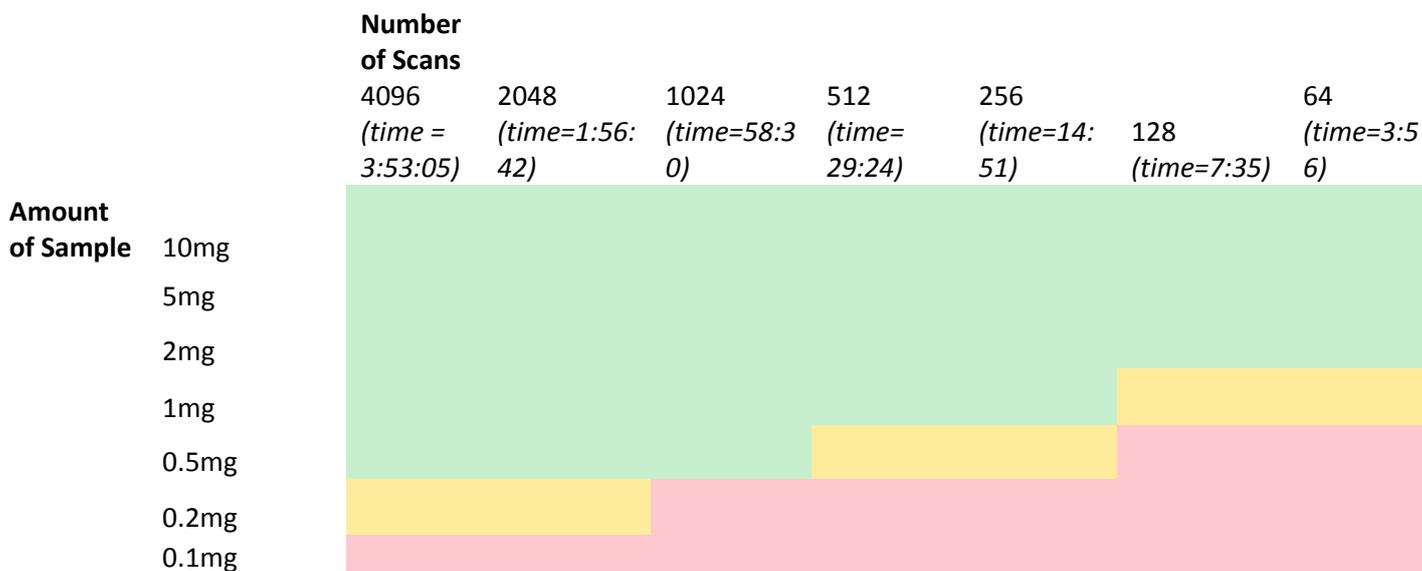


DEPT-135 sensitivity

Nano400 BBI DEPT-135 Sensitivity with artemisinin in 0.5ml CDCl₃ (ICON NMR - C13DEPT135p (dept135) NS=64; d1 =2; Time 3:51)

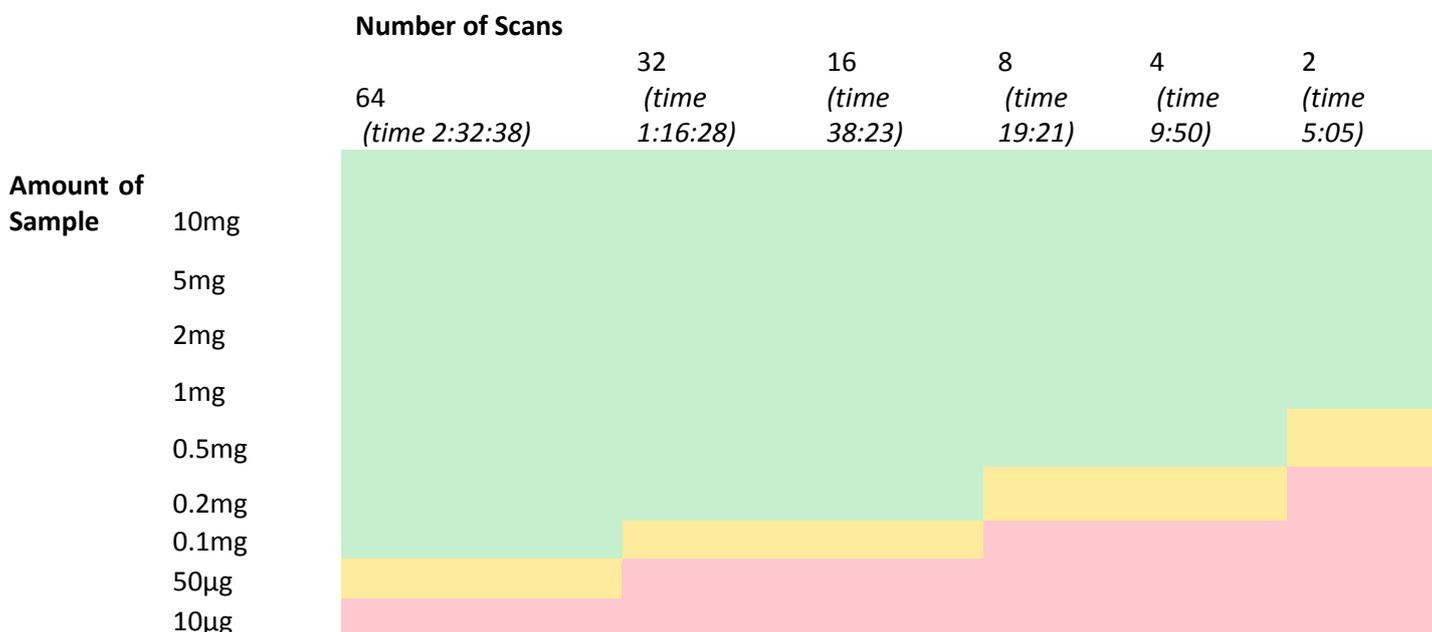


DPX400 BBFO DEPT-135 Sensitivity with artemisinin in 0.5ml CDCl₃ (ICON NMR - C13DEPT135p (dept135) NS=64; d1 =2; Time 3:56)

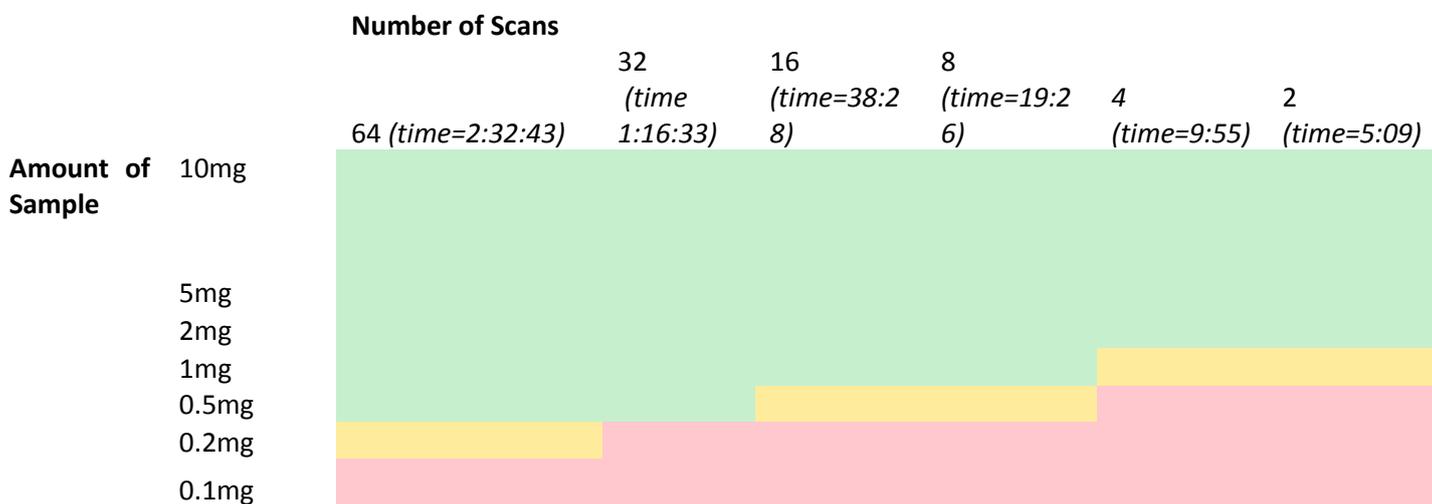


EDITED-HSQC sensitivity

Nano400 BBI Edited-HSQC Sensitivity with artemisinin in 0.5ml CDCl₃ (ICON NMR - HSQCEDETGP (hsqcedetgp) NS=2; d1=1; td(F1)=128 Time 5:05)

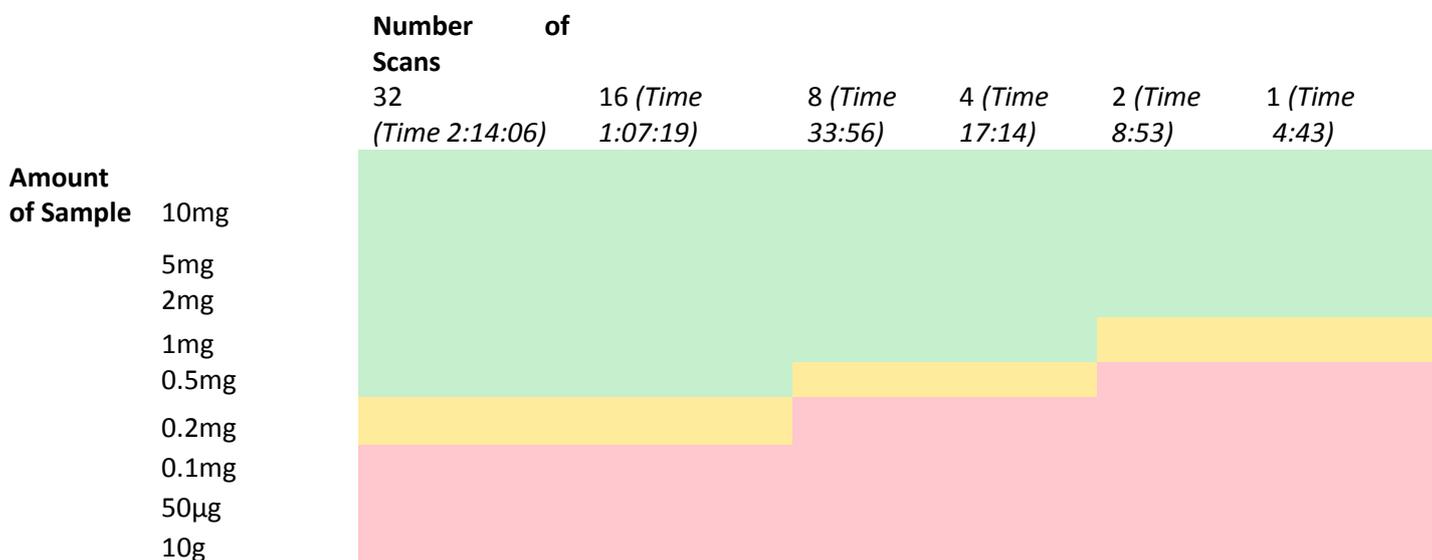


DPX400 BBFO Edited-HSQC Sensitivity with artemisinin in 0.5ml CDCl₃ (ICON NMR - HSQCEDETGP (hsqcedetgp) NS=2; d1=1; td(F1)=128 Time 5:09)



HMBC sensitivity

Nano400 BBI HMBC Sensitivity with artemisinin in 0.5ml CDCl₃ (ICON NMR - HMBCGPND (hmbcgpnd) NS=1; d1 =1.5; td(F1)=128; Time 4:43)



DPX400 BBFO HMBC Sensitivity with artemisinin in 0.5ml CDCl₃ (ICON NMR - HMBCGPND (hmbcgpnd) NS=1; d1 =1.5; td(F1)=128; Time 4:48)

