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1 Volumetric  $^{23}\text{Na}$  Single and Triple-Quantum Imaging at  
2 7T: 3D-CRISTINA

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7

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15

16

17 **ABSTRACT**

18 **PURPOSE** To measure multi-quantum coherence (MQC)  $^{23}\text{Na}$  signals for  
19 noninvasive cell physiological information in the whole-brain, the 2D-CRISTINA  
20 method was extended to 3D. This experimental study investigated the use and results  
21 of a new sequence, 3D-CRISTINA, on a phantom and healthy volunteers.

22 **METHODS:** The 3D Cartesian single and triple-quantum imaging of  $^{23}\text{Na}$  (3D-  
23 CRISTINA) was developed and implemented at 7T, favoring a non-selective volume  
24 excitation for increased signal-to-noise ratio (SNR) and lower energy deployment than  
25 its 2D counterpart. Two independent phase cycles were used in analogy to the 2D  
26 method. A comparison of 6-steps cycles and 12-steps cycles was performed.

27 We used a phantom composed of different sodium and agarose concentrations, 50  
28 mM to 150 mM  $\text{Na}^+$ , and 0–5 % agarose for sequence validation. Four healthy  
29 volunteers were scanned at 7T for whole brain MQC imaging. The sequence 3D-  
30 CRISTINA was developed and tested at 7T.

31 **RESULTS:** At 7T, the 3D-CRISTINA acquisition allowed to reduce the TR to 230 ms  
32 from the previous 390 ms for 2D, resulting in a total acquisition time of 53 min for a  
33 3D volume of 4x4x8 mm resolution. The phase cycle evaluation showed that the 7T  
34 acquisition time could be reduced by 4-fold with moderate single and triple-quantum  
35 signals SNR loss. The healthy volunteers demonstrated clinical feasibility at 7T and  
36 showed a difference in the MQC signals ratio of White Matter (WM) and Grey Matter  
37 (GM).

38 **CONCLUSION:** Volumetric CRISTINA multi-quantum imaging allowed whole-brain  
39 coverage. The non-selective excitation enabled a faster scan due to a decrease in  
40 energy deposition which enabled a lower repetition time. Thus, it should be the  
41 preferred choice for future in vivo multi-quantum applications compared to the 2D

42 method. A more extensive study is warranted to explore WM and GM MQC  
43 differences.

44 **Keywords:** sodium MRI, sodium triple-quantum imaging, whole-brain imaging

45

## 46 1 INTRODUCTION

47 Recently we proposed a 2D multi-quantum coherence (MQC) imaging method for  
48  $^{23}\text{Na}$  at 7T, CRISTINA [1], to fully exploit the potential of the spin 3/2 nuclei.

49 Sodium MRI is of interest for its noninvasive cell physiological information which are  
50 connected to multiple pathologies [2]–[10] and therefore could provide improved  
51 tissue characterization. MQC imaging focus lays on the joint measurements of single  
52 quantum (SQ) signal, for sodium concentration quantification, and triple quantum  
53 (TQ) signal, for information about the direct environment of the sodium nuclei, which  
54 differs from microscopic environment density and arises from the mechanism of  
55 biexponential relaxation.

56 The typical MQC three-pulses sequence leverages repetitions with a phase-cycling  
57 scheme for each quantum signal to follow different coherence pathways. CRISTINA  
58 provided an analytical understanding of phase cycling options, leading to an optimal  
59 phase-cycling design for simultaneous measurement of SQ and TQ coherence  
60 signals robust to  $B_0$  inhomogeneity, compared to the state-of-the-art SISTINA  
61 sequence that kept a bias due off-resonance phase accrual. The method consists of  
62  $2 \times 6$  steps phase cycles with a phase-increment of  $\pi/6$ . In the first cycle, the start  
63 phase for the first pulse is  $\pi/2$ , and the phase difference between the first and second  
64 pulse is also  $\pi/2$ . The second cycle has a start phase of 0 (first pulse) and a phase  
65 difference of 0 between the first and second pulse.

66 The experimental results were limited so far to elongated acquisitions for a single 2D  
67 plane [1]. The purpose of this study is to communicate practical benefits from the  
68 development of volumetric 3D-CRISTINA for 7T.

69 So far, the three pulses [11], multi-echo 2D-CRISTINA sequence was lacking a  
70 volumetric acquisition, needed for improved resolution, an extended field of view, and  
71 partial volume effects reduction to reach towards larger scale in vivo explorations.

72 Further, the long acquisition times at 7T of up to an hour mainly due to an elongated  
73 repetition time (TR) of 390 ms from specific absorption rate (SAR) restrictions, with  
74 conservative 12-step sampling and 10-fold averaging for a single slice was too long  
75 for multi-slice acquisition. The long TR was needed to reduce the SAR of the sinc-  
76 shaped, slice selective excitation.

77 A barrier of  $^{23}\text{Na}$  MRI is the low SNR and resulting long acquisition times which is why  
78 the higher field strength of 7T was favoured due to the supralinear rise of SNR with  
79 the static magnetic field  $B_0$ . [12] The purpose of this paper is to present 3D-CRISTINA  
80 feasibility at 7T. A phase cycle optimization led to the decrease of the total  
81 measurement time from 66 min to 32 min (phantom) and from 53 min to 13 min (in  
82 vivo). With 3D-CRISTINA, 3D single and triple quantum Images can be obtained  
83 simultaneously, within a reasonable acquisition time at 7T. The time efficiency of 3D-  
84 CRISTINA is validated on phantom measurements and MQC images are shown in  
85 vivo on four healthy volunteers.

86

## 87 2 MATERIAL AND METHODS

### 88 2.1 SEQUENCE DEVELOPMENT

89 The 2D-CRISTINA sequence [1] (**Figure 1 a**) was modified for the 3D-CRISTINA  
90 sequence (**Figure 1 b**) to feature a volumetric, multi-echo acquisition for joint multi-  
91 quantum signals and tissue sodium concentration (TSC) characterization. 3D-  
92 CRISTINA allows for a non-selective excitation to be employed with 3D Cartesian k-  
93 space sampling. CRISTINA used an optimal phase-cycle scheme of  $2 \times 6 = 12$  steps  
94 with 1) first pulse start phase of  $\pi/2$ , a phase difference between the first and second  
95 pulse of  $\pi/2$  and 2) first pulse start phase of 0 and phase difference between first and

96 second pulse also 0 with a phase increment of  $\pi/6$  was acquired. A detailed  
97 description about the underlying theory and choice can be found here: [1].  
98

99 *2.2 EXPERIMENTAL VALIDATION*

100 The sequence was tested on an agarose phantom on a 7T scanner (Siemens  
101 Healthcare, Magnetom 2<sup>nd</sup> generation whole body research system, Erlangen,  
102 Germany) with a 1Tx/1Rx dual-tuned <sup>1</sup>H/<sup>23</sup>Na birdcage head coil (Rapid, Biomedical).  
103 The phantom consisted of 9 tubes (14 and 40 mL) with varying concentrations of  
104 agarose (0-5 %) and sodium (50-154 mM) (see **Figure 2 A**).

105 Signal-to-noise ratio (SNR) was evaluated as the mean signal in a region of interest  
106 (ROI) covering the brain over the standard deviation of the signal in a background  
107 ROI outside of the brain. A correction factor of 0.655 was applied to account for the  
108 Rayleigh distribution of background noise. SNR of the two 40 mL tubes with 100 mM  
109 of sodium and 4 and 5 % agarose was compared between the 3D-CRISTINA and the  
110 2D-CRISTINA images as previously described [1] for each echo time. The SNR was  
111 further corrected for the voxel size to evaluate SNR variation associated with 3D  
112 excitation.

113 The dataset was further downsampled along the phase cycle dimension to investigate  
114 optimal choice for phase cycling options to further reduce acquisition time. Detailed  
115 sequence parameters are listed in **Table 1**. The vendor internal shimming procedure  
116 was repeated at least three times, carefully checking the frequency offset to be zero  
117 before each <sup>23</sup>Na scan. An incremental free-induction decay-based B1+ calibration  
118 was carried out before each scan to calculate the exact reference voltage for the 90°  
119 pulses.

120



<b>3D_CRISTINA PARAMETERS</b>	<b>Phantom</b>	<b>In vivo volunteers (1- 4)</b>
<b>matrix size</b>	48x48x14x20x24	32x32x12x20x24
<b>size after zero filling</b>	256x256x14x20	128x128x12x20
<b>base resolution</b>	48	32
<b>FOV</b>	200 mm	250mm
<b>resolution</b>	4x4x8 mm <sup>3</sup>	7.8x7.8x15 mm <sup>3</sup>
<b>1<sup>st</sup> pulse duration</b>	1800 $\mu$ s	1800
<b>2<sup>nd</sup>/3<sup>rd</sup> pulse duration</b>	1000 $\mu$ s	1000
<b>TE<sub>1</sub>, <math>\Delta</math>TE</b>	1.6 ms	1.6 ms
<b>TR</b>	160 ms	230 ms
<b>Bandwidth</b>	400 Hz/px	400 Hz/px
<b>Phasecycles</b>	2	2
<b>total scan time</b>	2x32.8 min = 66 min	2x26.7 min= 53 min
<b>xRef Voltage</b>	406 V	438 V, 452 V, 415 V, 437 V

121

122 *Table 1:* 3D Sequence Parameters at 7T. In vivo, the TR varied from the first to the second  
123 volunteer due to a higher reference voltage and SAR restriction. The TR was fixed to a higher  
124 parameter for the latter two volunteers. Further parameters which did not change between  
125 phantom and in vivo scans were: ( $\Delta\phi = 30^\circ$ ) resulting in a phase cycle of 12 points, mixing  
126 time = 100  $\mu$ s, evolution time = 10 ms, number of echoes = 20, asymmetry of echo = 0.8. Slice  
127 thickness 3D = 15 mm (in vivo), 8 mm (phantom).

128

129 **2.3 IN VIVO VALIDATION**

130 Four healthy volunteers (1 woman, 3 men,  $21 \pm 0.5$  years old) were recruited after  
131 providing informed consent according to the Declaration of Helsinki. The study was  
132 approved by the local ethical committee. Four additional 60 mL phantom vials were  
133 placed next to the head for sodium quantification, containing 5% and 2% agarose  
134 each with 100 mM and 50 mM  $\text{Na}^+$  concentration. The phantoms were fixed to the  
135 subjects' head with an elastic band. Additionally, to achieve TSC quantification the  
136 cerebrospinal fluid (CSF)  $\text{Na}^+$  literature value of 145 mM was used [13]. For one  
137 healthy volunteer we retrospectively undersampled the data to virtually reduce the  
138 measurement time according to the phantom 7T data.

139

#### 140 *2.4 DATA RECONSTRUCTION AND FIT*

141 In analogy to the data reconstruction for the 2D-CRISTINA sequence, the  
142 reconstruction was extended to 3D including the additional slice dimension. A 3D B0  
143 map was calculated from the first two echoes of the CRISTINA phase data to map  
144 the signal off-resonances for combination of the two phase cycles ( $\xi_1 = \pi/2$  and  $\xi_2 =$   
145  $0$ ) according to the method of *Fleysher et al.*[14] The voxel-wise multiparametric fit  
146 was updated to perform a joint fit of SQ and TQ signal, using GlobalSearch and  
147 fmincon solver in Matlab (R2019a, Mathworks, Natick, MA, USA), providing  $T2^*$ slow  
148 and  $T2^*$ fast maps. In the occurrence of one aberrant  $T2^*$  value, the signal fit was  
149 reverted to a mono-exponential decay (as in the case of pure saline). The cost  
150 function of the fit was implemented to weight the TQ signal first echoes higher than  
151 the later echoes and the SQ signal. The first echoes were chosen empirically to range  
152 from one to eleven to follow the characteristic curve of the TQ signal (see **Figure 1**)  
153 and to weight down the later echoes which have lower SNR (see Eq.[1-3]).

154

155

$$156 \quad f_{fitTQ_{-}(i)(x)} = A_{TQ} \left( \exp\left(-\frac{TE}{T2s^*}\right) - \exp\left(-\frac{TE}{T2f^*}\right) \right) \exp\left(-\frac{\tau1}{T2s^*}\right) + DC_{TQ}; i = 1, 2^{(*)}$$

157 [1]

$$158 \quad f_{fitsQ}(x) = \left( A_{SQ1} \exp\left(-\frac{TE+\tau1+\tau2}{T2s^*}\right) + A_{SQ2} \exp\left(-\frac{TE+\tau1+\tau2}{T2f^*}\right) \right) \exp\left(-\frac{TE+\tau1+\tau2}{T2s^*}\right) + DC_{SQ} \quad [2]$$

$$159 \quad L2(x) = w1 * \Sigma \left( TQ_{data}(TE_{1:TE_{11}}) - f_{fitTQ_{-}1}(x) \right)^2 + w2 * \Sigma \left( TQ_{data}(TE_{12:TE_{21}}) - f_{fitTQ_{-}2}(x) \right)^2 +$$

$$160 \quad w2 * \Sigma \left( SQ_{data} - f_{fitsQ}(x) \right)^2 \quad (**) \quad [3]$$

161

162 The variables in Eq. [1-3] are:163  $A_{TQ}$  = triple quantum signal amplitude,  $A_{SQ1}$  = single quantum amplitude slow term,164  $A_{SQ2}$  = single quantum amplitude fast term,  $DC_{TQ,SQ}$  = DC offset to account for noise,165 TE = echo time,  $T2s^*$  =  $T2^*$ slow,  $T2f^*$  =  $T2^*$ fast,  $\tau1$  = evolution time,  $\tau2$  = mixing time.166 (\*) The TQ fit was split into two parts  $f_{fitTQ_{-}1}$  and  $f_{fitTQ_{-}2}$ : 1) data for echoes 1 to 11 that

167 includes the expected TQmax signal and 2) data from echo 12 to the latest echo,

168 after the expected TQmax signal, to allow for different weighting in the cost function

169 L2.

170 (\*\*)  $w1, w2$  = weightings. The weightings were empirically optimized to  $w1=0.8$  and171  $w2=0.2$ . The fit routine included both  $T2^*$ fast and  $T2^*$ slow with boundary settings172  $T2^*$ fast: [2 ms; 10 ms] and  $T2^*$ slow [8 ms; 55 ms].

173

174

## 175 2.5 PHASE CYCLE OPTIMIZATION

176 The phase increment of the chosen phase cycle was initially chosen with  $30^\circ$  to  
177 conform to Nyquist criterion of sampling the signal along the phase axis with twice the  
178 frequency of interest (i.e. 12 steps cycles). The minimum number of steps is 6 [1],  
179 with corresponding phase increment of  $60^\circ$ , for the TQ acquisition and a decrease to  
180  $30^\circ$  increment results in a 2-fold measurement time increase. As an optimization of  
181 efficacy, 12-step ( $\Delta\phi = 30^\circ$ ) datasets were subsampled into the 6-step subset of  $\Delta\phi$   
182  $= 60^\circ$  with the initial start phase of  $\phi_1(t = 0) = 90^\circ$ . Additionally, two averages were  
183 used to obtain higher SNR within allotted time. The initial 12-steps 2-averages results  
184 were compared to the minimal 6-steps 1-average subset results in terms of SQ and  
185 TQ SNR, both in vitro and in vivo.

186

## 187 2.6 DATA EVALUATION

188 We calculated the SNR of SQ and TQ along echo time within the whole image within  
189 the 6-pixel eroded body mask and the standard deviation of the noise in the  
190 background. Additionally, SNR was computed in the four 4 % and 5 % agar phantom  
191 vials using centered circular regions of interest, 2 pixels smaller than the outline, to  
192 avoid partial volume effects. The fit result contained  $T2^*$ slow and  $T2^*$ fast maps and  
193 the fitted amplitudes for  $A_{SQslow}$ ,  $A_{SQfast}$ ,  $A_{TQ}$ . The fit was performed for a single slice  
194 chosen at the center of the 3D datasets, respectively.

195

196

197

## 198 3 RESULTS

199

### 200 3.1 PHANTOM RESULTS

201 The experimental results from 3D-CRISTINA with the phantom are given in **Figure 2**  
202 for the five central slices of each dataset. Compared to previous 2D results from [1],  
203 SNR dropped by  $2.27 \pm 0.46$ -fold with chosen acquisition parameters  
204 (**Supplementary Material Figure 1**). When correcting for the voxel size  
205 ( $5.21 \times 5.21 \times 20$  mm<sup>3</sup> in 2D to  $4 \times 4 \times 8$  mm<sup>3</sup> in 3D), which led to a global SNR loss of  
206 4.24-fold, the actual SNR gain for moving from 2D to 3D was  $4.24/2.27 = 1.87$ -fold  
207 improvement. With 3D-CRISTINA, the three phantom vials: 2, 4, and 5, with an agar  
208 concentration of 4%, 4% and 5% can be clearly distinguished from the remaining vials  
209 on the TQ image. The relationship of TQ signal to agar concentration of the different  
210 > 40 mL vials, (vials 8,9 were not included due to their sizes) was linear with  $R^2 =$   
211 0.987 (**Figure 2 d,e**). The phantom vial 7 was disregarded due to the low sodium  
212 concentration leading to a limited SNR in SQ and TQ images.

### 213 3.2 FIT RESULTS

214 For the center slice, a multiparameter fit was performed (**Figure 3**) along echo time  
215 (**Equation 1-3**). The average  $T2^*$  values were evaluated within each phantom vial  
216 and resulted in a mean  $T2^*_{fast}$  and  $T2^*_{slow}$  value of the phantom vials 2, 4, 5 and 8  
217 which contained 4% and 5% of agarose, of ( $T2^*_{fast}: 5 \pm 2$  ms;  $T2^*_{slow}: 34 \pm 9$  ms). For  
218 vials 3, 6, and 9, which contained 2% agarose, we obtained:  $T2^*_{fast}: 8 \pm 3$  ms;  $T2^*_{slow}: 50 \pm 5$  ms. Vial 7 with 4% agar and 50 mM resulted in  $T2^*_{fast}$  of  $6 \pm 3$  ms and  $T2^*_{slow}$  of  
219  $44 \pm 15$  ms. The liquid saline solution resulted in  $T2^*: 34 \pm 11$  ms.

### 221 3.3 PHASECYCLE OPTIMIZATION RESULTS

222 To virtually cut down measurement time by 4x (from 53 min to 13 min in vivo, and 64  
223 min to 16 min in the phantom), we compared the initial 12-step phase cycle ( $\Delta\phi =$   
224  $30^\circ$ ) with two averages to a single cycle with  $\Delta\phi = 60^\circ$ . The comparison of SQ and  
225 TQ signals can be seen in **Figure 4** on phantom. By taking only a quarter of the data,  
226 the SQ SNR decreased by  $39\pm 5\%$  and the TQ SNR decreased by  $31\pm 2\%$ . The  
227 limited SNR drop (compared to expected 50 %) reflected the influence of samples  
228 position within the phase cycle. The extra 6 steps with initial phases  $120^\circ$ ,  $180^\circ$ ,  $240^\circ$ ,  
229  $300^\circ$  and  $360^\circ$  are not participating in the TQ signal build-up as they correspond to  
230 zero-crossing of the TQ signal. Additionally, these points contributed sub-optimally to  
231 SQ signal considering their relative amplitude in the SQ coherence profile, shaped by  
232 a sinus wave. **Figure 5** shows the results on in vivo data.

### 233 3.4 INVIVO HEALTHY VOLUNTEER RESULTS

234 **Figure 6** showed the capacity of 3D-CRISTINA to visualize sodium SQ and TQ signals  
235 across the whole brain, even in regions of  $B_0$  inhomogeneity. TQ signal did not exhibit  
236 structured variations across slices, while SQ signal reflects the different  
237 concentrations of sodium between WM, GM and CSF. SQ results from the four  
238 healthy volunteers (**Figure 7**) showed consistent signal intensity distribution with  
239 conventional single pulse Na-MRI sequences [15]. From ROI measurements, mean  
240 TSC values showed an increase sodium concentration from WM to GM to CSF ( $52.9$   
241  $\pm 4.1$ ,  $96.6 \pm 22.0$  and  $170.0 \pm 23.9$  mM respectively). Fitted signals TQ/SQ ratio  
242 exhibited a corresponding decrease from  $0.28 \pm 0.07$  (WM) to  $0.14 \pm 0.04$  (GM) and  
243  $0.10 \pm 0.03$  (CSF), reflecting a relatively homogeneous measured TQ signal in the  
244 whole brain. **Figure 7** showed TQ signal variations were much lower than SQ signal  
245 variations in all volunteers. Consequently, the TQ/SQ ratio showed strong differences  
246 between gray and white matter. White matter exhibited low SQ signal, levelled TQ

247 signal and thus higher TQ/SQ ratio, suggesting that despite a lower sodium  
248 concentration, a stronger bi-exponential signal separation derived from WM molecular  
249 environment. On the contrary, GM exhibited increased SQ signal from higher sodium  
250 concentrations, but TQ signal on the levels of WM. This relationship confirms a  
251 different balance of exponential components, with a larger component for the longer  
252 T2\* decay [16]. Expectedly, CSF exhibited a negligible TQ/SQ ratio.

253

#### 254 4 DISCUSSION

255 In this study, 3D-CRISTINA demonstrated consistent MQ images at 7T in a dedicated  
256 agar phantom. The non-selective excitation allowed to improve the spatial resolution  
257 and decrease the acquisition time, also combined with an increase in SNR.  
258 Furthermore, the choices for phase cycling were investigated and optimized  
259 retrospectively. The conservative phase cycling of 12 ( $\Delta\phi = 30^\circ$ ) steps was evaluated  
260 against 6 steps ( $\Delta\phi = 60^\circ$ ) options in the well-controlled phantom experiments. An  
261 acquisition time reduction from 66 min to 16.4 min in the phantom was possible  
262 without moderate SNR loss in the TQ signal. The phantom results motivated in vivo  
263 exploration in healthy volunteers in the brain where we found consistent results across  
264 slices and subjects. In vivo, total scan time reached 53 min for a whole brain  
265 coverage. The same 4-fold time reduction in vivo led to limited SNR loss, motivating  
266 the use of the 6-steps cycles only, with more averages if examination time permits it.  
267 Nevertheless, further developments could improve resulting SNR within limited time,  
268 utilizing multi-channels coil hardware and/or advanced image reconstruction  
269 techniques (compressed sensing or deep learning techniques [17]–[19]).

270

271 For tissue sodium concentration quantification in vivo, the SQ signal at the first echo  
272 time ( $TE_1=1$  ms) was employed instead of the more accurate the fitted SQ signal at  
273  $TE = 0$  ms to avoid potential bias introduced by the bi-exponential fitting. Additionally,  
274 TSC references were defined by the CSF and the 100 mM vial instead of the 50 mM  
275 phantom vial for accuracy. Indeed, external vials placed at the edges of the coil might  
276 experience inhomogeneous  $B_1+$  that penalizes lower signal estimation.

277 The in vivo data exhibited a difference of TQ/SQ ratio between GM, WM and CSF in  
278 the four volunteers. Considering WM contains myelinated structured axons while GM  
279 is made up of cell bodies, the molecular environment of sodium atoms in these  
280 compartments is expected to strongly differ. However, CSF is expected to present a  
281 very low TQ/SQ ratio from relatively free molecular environment. Our results suggest  
282 current noise levels could bias proper estimation of very low TQ signal in CSF.  
283 Nevertheless, a larger study is warranted to confirm these initial results based on only  
284 four volunteers. Further study microstructure similar to Kolbe et al. [20] could be  
285 envisioned combining 3D-CRISTINA with DTI to map these differences in regard to  
286 the brain architecture.

287 One limitation of this study was the loss of SNR from too ambitious spatial resolution.  
288 The reduction of the voxel size was too drastic compared to the 1.87-fold SNR  
289 improvement provided by 3D-CRISTINA. A relatively lower resolution would have  
290 better shown 3D-CRISTINA benefits. Another limitation is the limited number of in  
291 vivo data for justified GM and WM quantification. An additional T1-weighted sequence  
292 (eg. the MP2RAGE sequence [21]) acquisition would have helped to provide a GM  
293 and WM segmentation. But the MP2RAGE sequence was designed to be employed  
294 with a multi-channel receiver coil array, requiring a change of coil which was  
295 incompatible with this study [22]. Another clear limitation is the low SQ and TQ signal  
296 of phantom vial 7 (4 % agar and 50 mM  $^{23}\text{Na}$ ), further work is needed with



297 standardized fabricated phantoms to explore limitations and rule out phantom ageing  
298 problems.

299 Further, signal variations in TQ images need to be explored to rule out variations due  
300 to B1+ field inhomogeneities. With our current coil, we measured flip angles deviation  
301 up to 10% within in the brain, which remains an acceptable precision. In addition, a  
302 comparison of healthy volunteers to pathological indications is necessary to evaluate  
303 the sensitivity of the TQ signal obtained by this sequence.

304 We reported an updated processing routine that is jointly fitting the SQ and TQ data  
305 to obtain T2\* fast and slow parameters. We chose to weigh down the later TQ signal  
306 after TE>15ms in the loss function. The earlier part (TE<15ms) contains the  
307 characteristic curve and should be more accurate in the determination of T2\* values.  
308 Nevertheless, it must be emphasized that the cost function weights were chosen  
309 empirically. The reported T2\* values align with the literature [13]. We limited our  
310 conclusions for in vivo exploration based on TQ, SQ signals and their ratio, which are  
311 more consistent measures for tissue characterization than the higher complexity  
312 CRISTINA model.

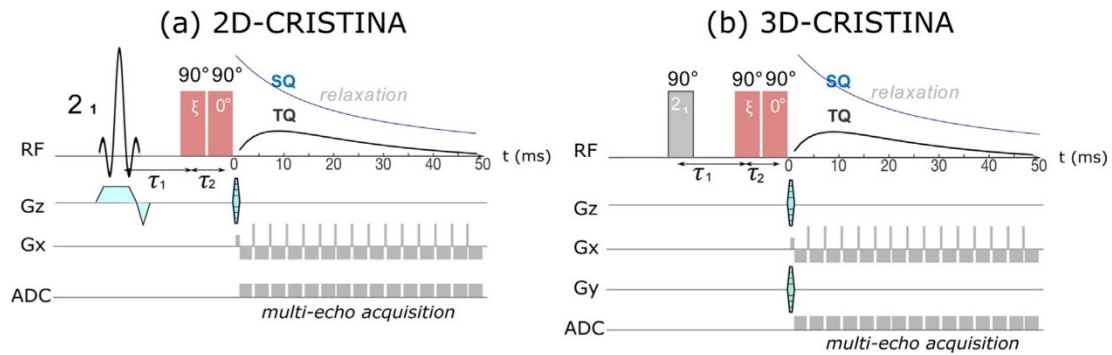
313

314 While 2D-CRISTINA focused on phase cycling schemes and probing multi-quantum  
315 pathways with accuracy and efficacy, 3D-CRISTINA considers practical MRI  
316 acquisition towards in vivo sodium multi-quantum imaging. In this study, we explored  
317 the feasibility of CRISTINA in the whole brain at 7T. Next, further work towards the  
318 more readily available clinical field strength of 3T is also warranted for more  
319 accessible multi-quantum imaging. This could extend the current state of the art in  
320 sodium imaging that relies up to date on the single quantum imaging for tissue sodium  
321 characterization with ultrashort echo time sequences [23], [24].

322

323 In conclusion, the presented technological advances enabled consistent sodium MQC  
324 imaging in acceptable time for research investigations, which offers the possibility to  
325 study ionic distribution and environment in neuropathologies such as multiple  
326 sclerosis, stroke or tissue degradation after radiation therapy in dedicated protocols.  
327 [25] This step is important for the active research field of  $^{23}\text{Na}$  imaging which is  
328 especially interested in the biochemical characterization of tissue.

## 329 LIST OF FIGURE CAPTIONS



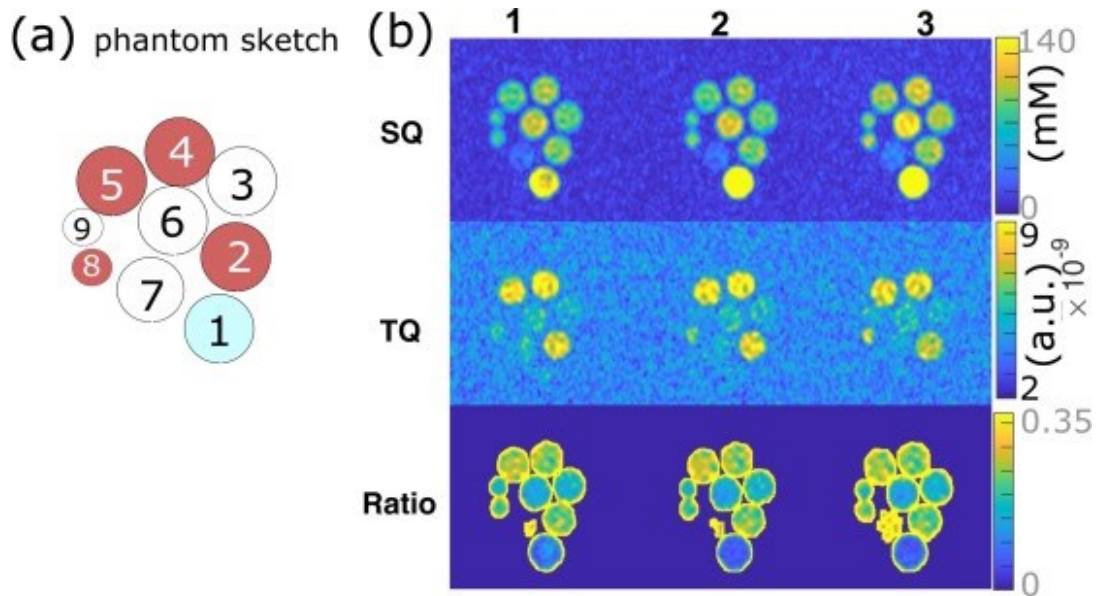
330

331 **Figure 1:** The previous 2D-CRISTINA sequence (a) featuring three pulses for multi-quantum

332 excitation, was extended to 3D-CRISTINA (b) by adding an additional phase encoding

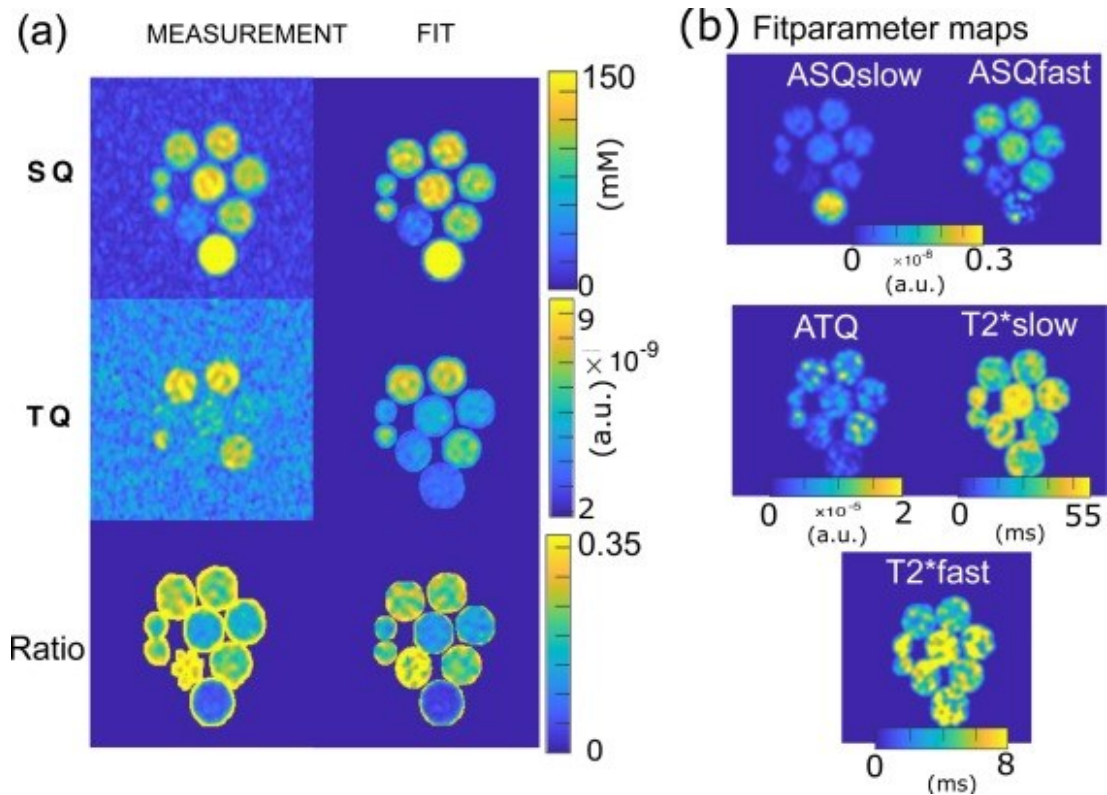
333 direction and substituting the slice selection by a non-selective excitation.

334



335

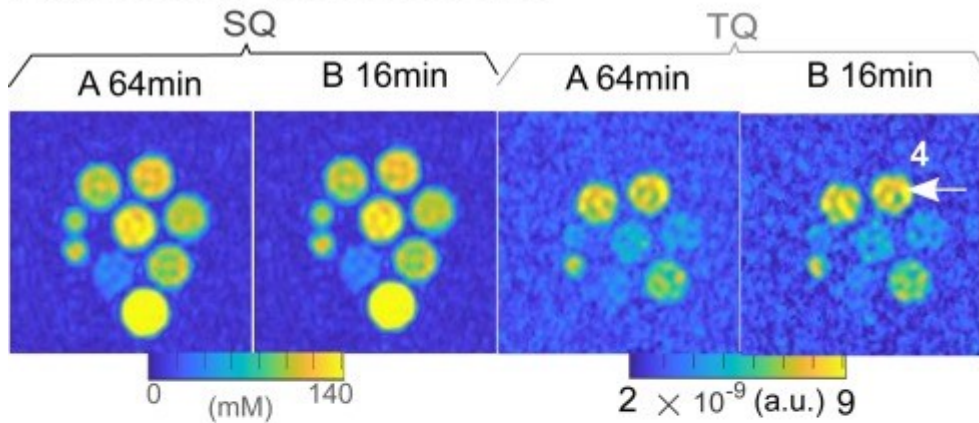
336 **Figure 2:** (a) Phantom design for subsequent sequence evaluation composed of nine tubes  
 337 with different agar (in %) and sodium concentration (in mM): 1) 0 %, 154 mM, 2) 4 %, 100 mM  
 338 3) 2 % agar, 100 mM 4) 4 %, 154 mM 5) 5 %, 154 mM 6) 2 % 130 mM 7) 4 % 50 mM 8) 4 %  
 339 100 mM 9) 2 %, 100 mM. Phantom vial 8 and 9 have a much smaller volume with 14 mL  
 340 compared to the 2-5 with 40 mL. The phantom vials indicated in red correspond to the high  
 341 agar vials containing >4 % agar. b) Phantom result of 3D-CRISTINA, SQ and TQ signal and  
 342 their ratio for 3 central slices each. (c) For the ratio versus agar concentration evaluation  
 343 without phantom 7-9 the result was:  $R^2 = 0.987$ ,  $p = 6e-5$ . (d) The SNR of TQ and SQ over  
 344 echo time shows the characteristic signal evolution respectively.



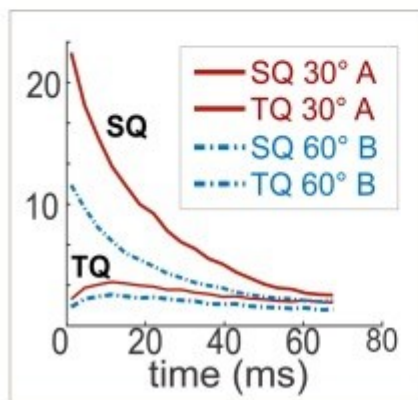
345

346 **Figure 3:** Fit result for the phantom dataset for a single slice at the center of the dataset. (a)  
 347 results with the measurement and fit data side by side. The fit was performed only within the  
 348 body mask therefore the Fit results and Ratios are zero in the background. (b) Resulting fit  
 349 parameter maps corresponding to Eq. 1-3. The liquid vial follows a mono-exponential decay  
 350 by comparing the signal amplitudes ASQslow and ASQfast maps. In the T2\* maps it can be  
 351 appreciated that the higher agarose phantoms show shorter relaxation times.

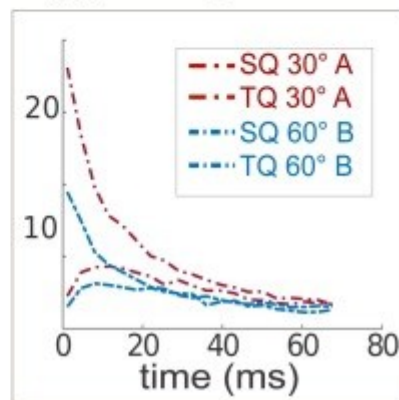
## (a) phantom measurements



## (b) SNR\_image

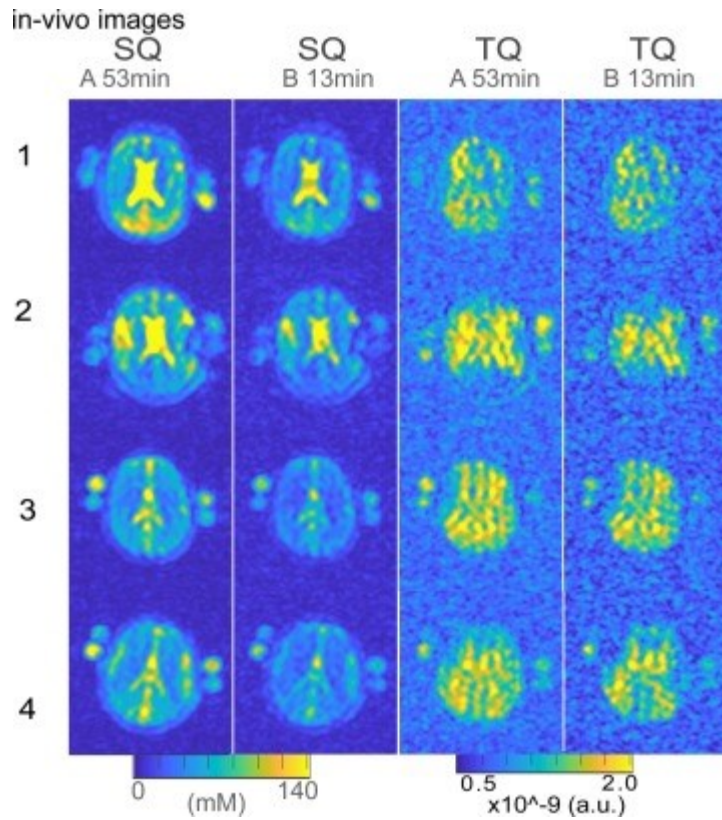


## (c) SNR\_vial 4



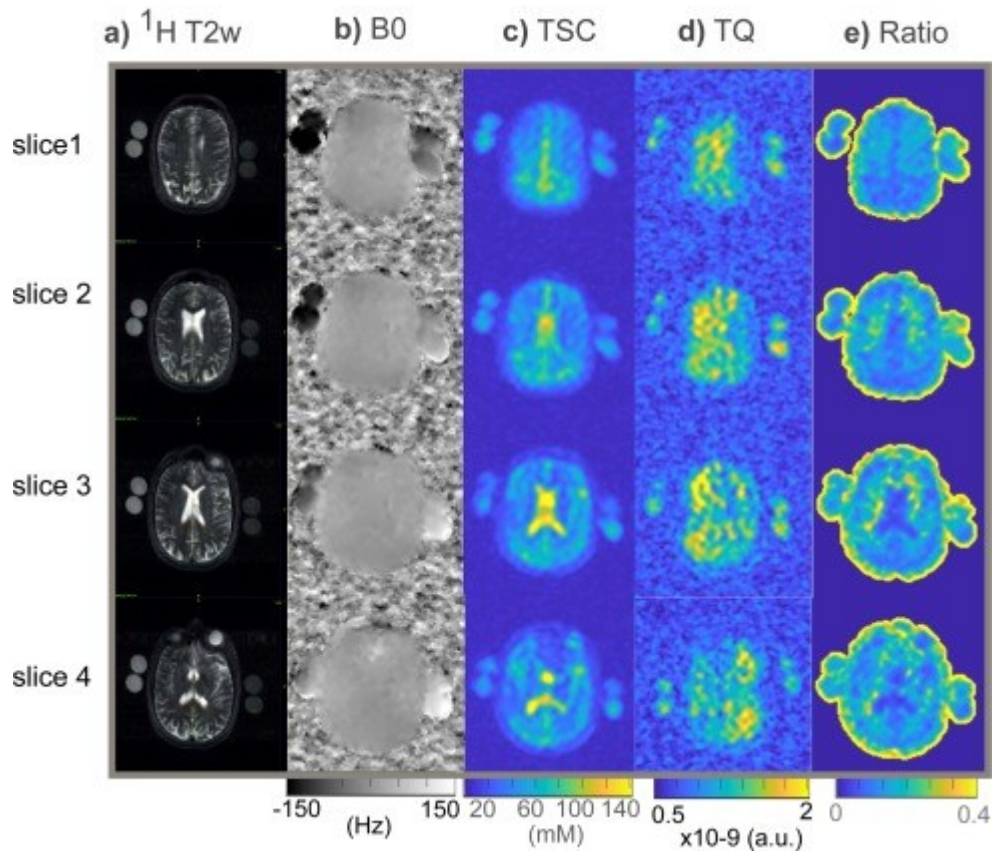
352

353 **Figure 4:** (a) phantom measurement comparison of TQ and SQ signal when doubling the  
 354 phase cycle increment. (A) 64min was acquired with a phase cycle increment of  $\Delta\phi = 30^\circ$  and  
 355 two cycles of each 6 steps. For (B) 16min, the increment was doubled to  $\Delta\phi = 60^\circ$ , without  
 356 averaging, acquiring only a fourth of the data. The SQ and TQ SNR for the whole image was  
 357 evaluated in (b). Due to cutting the amount of data the SNR is higher for A than for B, however  
 358 visibly the image quality is not compromised, confirming the possibility of a shorter  
 359 measurement time. (c) the SNR in vial 4 shows the signal comparison for A versus B  
 360 measurement time in the 4 % 154 mM vial.



361

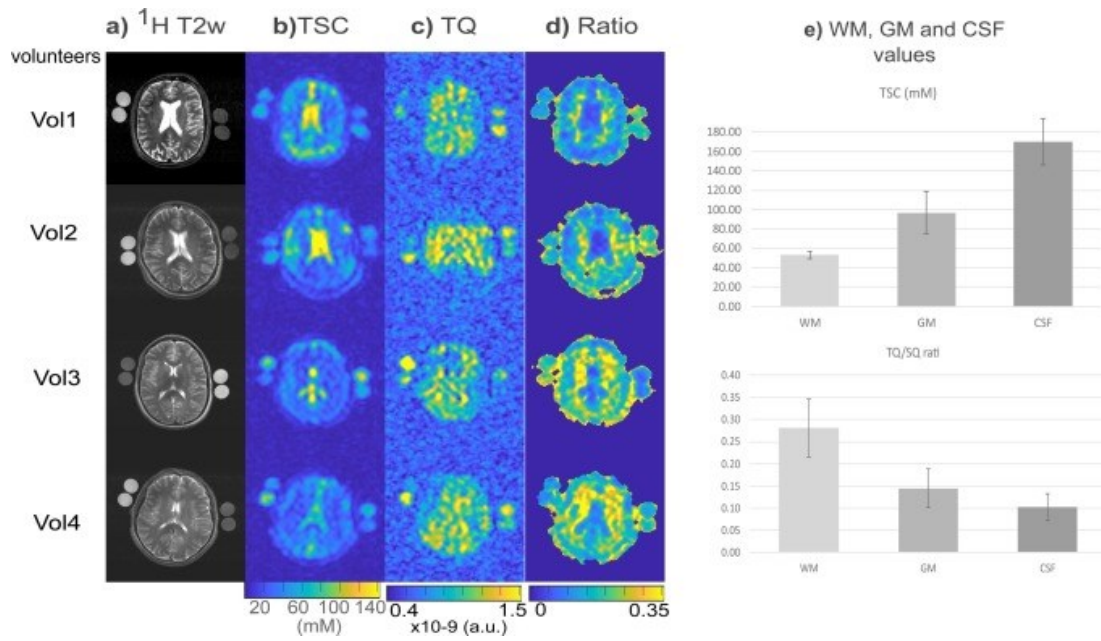
362 **Figure 5:** Visual Comparison of TQ and SQ signal when doubling the phase cycle increment,  
363 without averaging, in vivo for the 4 different healthy volunteers in analogy to the phantom  
364 measurement shown in Figure 4.

**In Vivo at 7T**

365

366 **Figure 6:** 7T in vivo results, different slices of volunteer 1. (A) T2w transversal  $^1\text{H}$  image  
 367 showing the corresponding slices. (B,C)  $^1\text{H}$ -T2w overlay with SQ and TQ signal to relate the  
 368 high intensity regions to the morphology better described by  $^1\text{H}$  images. (D) B0 maps  
 369 reconstructed from the multi-echo CRISTINA data, (E) SQ images quantified to Tissue Sodium  
 370 Concentration, (F) TQ images as well as the ratio of SQ and TQ signal in (G).





371

372 **Figure 7:** 7T in vivo results of the four volunteers demonstrated homogeneous TQ signal  
 373 within the brain, with occasional darker regions in the periphery. These signal losses might be  
 374 associated with the extra B1 sensitivity of TQ signal (to the power of 5). Interestingly, the  
 375 TQ/SQ ratio highlighted regions of white matter in all volunteers, and low ratio values in  
 376 cerebro-spinal fluid area.

377

378

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