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**A roadmap to high-resolution standard microcoil MAS NMR spectroscopy
for metabolomics**

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20 **Abstract**

21 The current microcoil probe technology has emerged as a significant advancement in NMR
22 applications to biological research of biofluids and continued to excel as a hyphenated tool
23 with other prominent microdevices opening many new possibilities in multiple omics fields.
24 However, this is contrary to biological samples like intact tissue or organism. This is due to
25 the considerable challenges of incorporating the microcoil in a magic-angle spinning (MAS)
26 probe without relinquishing the acquisition of high-resolution spectral data. Not until 2012, a
27 microcoil MAS probe had shown promises on profiling the metabolome in submilligram
28 tissue biopsy with spectral resolution *on par* with the conventional high-resolution MAS
29 (HR-MAS) NMR. This result had subsequently triggered a great interest in the possibility of
30 NMR analysis with microgram tissues and strived toward the probe development of 'high-
31 resolution' capable microcoil MAS NMR spectroscopy. This review gives an overview of the
32 issues and challenges in the probe development and summarizes the advancements toward
33 metabolomics.

34

35 Keywords: microcoil; magic-angle spinning; high-resolution; sensitivity; metabolomics;

36

37 **Selected Abbreviations**

38

39	χ	magnetic susceptibility factor
40	μ	micro
41	B	magnetic field
42	B_0	external applied magnetic field
43	B_1	transverse radiofrequency magnetic field
44	B_z	dipole field of a magnetic moment
45	DNP	dynamic nuclear polarization
46	FWHM	full-width at half-maximum
47	i	electrical current
48	HMQC	heteronuclear multiple quantum coherence
49	HR-MAS	high-resolution magic-angle spinning
50	rf	radiofrequency
51	SNR	signal-to-noise ratio

52

53 **Introduction**

54 Metabolomics is a significant omics field that studies the chemical fingerprints of small
55 molecule metabolites that responds to specific cellular processes. It plays a vital role in
56 untangling many complexities in life science. This is thanks to the vast advancement in
57 bioanalytical technology. *Ex vivo* NMR spectroscopy has found tremendous success due to its
58 simplicity and versatility in acquiring unbiased and rich metabolic information on diverse
59 biospecimens from biofluids to biotissues. In particular, High-Resolution Magic-Angle
60 Spinning (HR-MAS) – rapid sample spinning under a magnetic field – is an indispensable
61 technique in NMR studies of animal and human tissue.¹⁻⁷ This is attributed to its ability to
62 acquire superior spectral resolution data from heterogeneous specimens (*i.e.*, *on par* with the
63 liquid state NMR). However, despite its significant applications to NMR-based
64 metabolomics, the probe technology of HR-MAS has remained nearly the same in the past
65 decades. The latest substantial advancement would be the fully automated HR-MAS probe
66 (iProbe™) in 2018.⁸ In contrast, other bioanalytical technologies like mass spectrometry
67 (MS),⁹ magnetic resonance imaging,¹⁰ and positron emission tomography¹¹ continue
68 advancing. There is a common conception that the current number of MS-based metabolomic
69 studies has overtaken NMR.¹² A literature search in Web of Science™ shows a nearly 2:1
70 ratio between MS and NMR metabolomics. Therefore, for NMR spectroscopy to continue to
71 be a frontline *ex vivo* analytic platform, new technologies and concepts should be explored
72 entirely to address the limitations.

73

74 Presently, there is a general tendency to develop biotechnology toward microscopic
75 specimens. The notion is to advance the underlining omics understanding at a single-cell (or
76 even subcellular) level.¹³ One weakness in NMR spectroscopy is the inherent low detection
77 sensitivity. The HR-MAS application generally requires 5–10 mg samples to compensate for
78 the low sensitivity. This hinders the possibility of analyzing μg samples. Indeed, using a high
79 magnetic field is an approach to enhance detection sensitivity. However, the running cost is
80 exponential with the field. A cost-effective method is to utilize a miniature radiofrequency
81 (rf) coil, so-called microcoil (μcoil), for optimizing the sample and coil volume ratio (*i.e.*,
82 filling factor). The prime concern is maintaining high spectral resolution data to allow in-
83 depth metabolic profiling. This is achieved in liquid-state NMR by surrounding the microcoil
84 with magnetic susceptibility-matched material to facilitate the field shimming¹⁴ or using a

85 zero-susceptibility μ coil.¹⁵ These adaptations have subsequently led to significant
86 advancements in microcoil applications to biofluid studies.¹⁶

87

88 The fact that nano-scale volume of tissue and cell can be optimally detected and analyzed
89 with a cost-effective approach makes μ coil an appealing technology. However, the μ coil
90 technology under MAS, or microcoil MAS, is lagging. This is due to the challenges of
91 implementing a stable μ coil that can endure a rapid sample spinning. The early designs
92 centered on implanting an independent microcoil onto a conventional MAS probe and
93 utilizing the existing stator to propel the sample spinning. The first microcoil under MAS was
94 introduced in 2006 with a piggyback design¹⁷ and was shortly followed by an inductive
95 spinning μ coil approach.¹⁸ These results had subsequently sparked the interest in the
96 commercial and academic groups and have resulted in multiple commercial probes with rotor
97 diameters ranging from 1-mm down to 0.5-mm.¹⁹⁻²¹ These state-of-the-art probes are
98 explicitly designed to spin mg-scale samples up to 100+ kHz to narrow the resonance
99 linewidth that arises from the nuclear spin networks. As a result, they have an immediate
100 impact on the MAS applications in many research fields of biosolids,²²⁻²⁸ including the study
101 of membrane proteins²⁹ and amyloid fibers.³⁰ However, the ultra-fast sample spinning is not
102 of concern in the metabolomic study of delicate samples like tissues and cells. It is considered
103 an unfavorable condition because of the enormous centrifugal force exerted on the sample. A
104 significant concern in designing a microcoil probe for metabolomics is its ability to acquire
105 sufficient spectral resolution data to unfold the dense metabolic ¹H resonance pattern and
106 permit thorough peak analyses for identification and quantification. Moreover, the ability to
107 acquire reproducible data is also a crucial factor. This depends on the stability of the probe
108 and the consistency of the sample preparation for the NMR measurements.

109

110 The possibility of ¹H NMR profiling μ g biopsy tissue under MAS condition was first
111 evaluated in 2012.³¹ It subsequently triggered the interest of striving for the possibility of
112 'high-resolution' standard microcoil MAS toward NMR-based metabolomics. Despite the vast
113 number of excellent review literature on HR-MAS NMR spectroscopy,¹⁻³ microcoil NMR,¹⁶
114 and microcoil MAS,²²⁻³⁰ there are none on the 'high-resolution' standard microcoil MAS.
115 Hence, this review aims to bridge the high-resolution microcoil technology under MAS
116 conditions with metabolomics and provides the fundamental basis for future advancements. It

117 summarizes the current developments, emphasizing our efforts from a nonexistence state to a
 118 beacon of possibility.

119

120 **NMR considerations**

121 NMR is a spectroscopic technique that characterizes the net magnetization \mathbf{M} induced in a
 122 sample exposed to an external field \mathbf{H}_0 , which can be described by a magnetic susceptibility
 123 factor χ , $\mathbf{M} = \chi\mathbf{H}_0$. The net magnetic field \mathbf{B}_0 in a sample exposed to \mathbf{H}_0 can be expressed as

$$124 \quad \mathbf{B}_0 = \mu_0(\mathbf{H}_0 + \mathbf{M}) = \mu_0\mu\mathbf{H}_0 \quad (1)$$

125 where μ_0 is a permeability of free space, and $\mu = 1 + \chi$ is the permeability of the sample. χ
 126 is a dimensionless quantity that indicates the extent of the magnetization \mathbf{M} in a sample under
 127 an external field. Several sources contribute to \mathbf{M} , which arise from the energy current within
 128 atoms, electrons, and nucleons. They are essentially responsible for an NMR resonance line
 129 intensity and shape. This review focuses only on the magnetizations that contribute to the
 130 resonance intensity and resolution. Note that in NMR and MRI, the external magnetic field is
 131 often denoted as B_0 rather than H_0 ; thereby, B_0 will be used hereafter.

132

133 **Sensitivity.** It is a hallmark problem for NMR and is attributed to the nuclear spin
 134 magnetization M_0 . At thermal equilibrium, M_0 can be expressed by the Boltzmann
 135 distribution, for 1/2-spin nuclei,

$$136 \quad M_0 = \frac{\gamma\hbar N}{2} P, \text{ where } P = \frac{N_\alpha - N_\beta}{N} \equiv \tanh\left(\frac{\gamma\hbar B_0}{2k_B T_s}\right) \quad (2)$$

137 where γ is the gyromagnetic ratio, \hbar ($h/2\pi$) is the Plank's constant, N is the total number of
 138 spins ($N_\alpha + N_\beta$), and P is the nuclear polarization, B_0 is the applied external magnetic field, k_B
 139 and T_s is the Boltzmann's constant and the sample temperature. The intensity of a detected
 140 signal is proportional to the polarization. For example, at a typical NMR field of 11.7 T at an
 141 ambient temperature of 300 K, only about 40 ppm of protons are polarized. Such small
 142 polarization is the very reason for the inherently low detection sensitivity in NMR.

143

144 Aside from M_0 , the signal intensity – quantified by a signal-to-noise ratio (SNR) – also
 145 depends on the instrumentations, which can be breakdown into a few distinct components:³²

$$146 \quad SNR \propto [\gamma B_0]_{\substack{\text{oscillation} \\ \text{frequency}}} \times [M_0]_{\text{magnetization}} \times [B_1/i]_{\substack{\text{coil} \\ \text{efficiency}}} \times \left[\frac{1}{\sqrt{T_c R_c}} \right]_{\substack{\text{thermal} \\ \text{noise}}} \quad (3)$$

147 The first two correspond to the oscillation and the polarized magnetization of the nuclear
 148 spin. The B_1/i term is defined as the transverse magnetic field B_1 per unit current associated

149 with the rf efficiency inside the coil volume. The last term is the thermal noise ascribed from
150 the coil's temperature T_c and resistance R_c . Essentially, the strategy for increasing the SNR is
151 by manipulating the individual components through physics and engineering. For example, a
152 common conception is to raise the external magnetic field B_0 to enhance the spin oscillation
153 and magnetization. Since the signal and noise increase as a square and a square root of
154 the B_0 field, therefore the overall SNR increases as $B_0^{3/2}$. The current highest commercial
155 NMR field is 28 T (1.2 GHz for ^1H).³³ Further increases are expected to be challenging and
156 expensive to overcome the issues of field homogeneity and stability.

157

158 Other approaches include manipulating the spin magnetization M_0 by polarization transfer. It
159 involves transferring the polarization from nuclei with high magnetic moment to lower
160 magnetic moment nuclei, offering a gain in sensitivity corresponding to the ratio of their
161 gyromagnetic ratios, $\gamma_{\text{high}}/\gamma_{\text{lower}}$. This is routinely applied in solid-state ^{13}C (or ^{29}Si) NMR
162 pulse experiments by polarizing the ^{13}C spins with the neighboring ^1H and can gain up to a
163 factor of ~ 4 ($\gamma_{^1\text{H}}/\gamma_{^{13}\text{C}}$). Similarly, the polarization transfer from the electron spin to nuclear
164 spin can have a tremendous gain (up to 10^4 – 10^5) because of the large Zeeman splitting in the
165 electron spin. Such transfer enters the regime of hyperpolarization, where the spin
166 polarization deviates from thermal equilibrium. This phenomenon between the electron and
167 nuclear is known as Dynamic Nuclear Polarization (DNP). Academic and commercial
168 researchers have invested tremendous efforts in integrating DNP with high-field NMR and
169 MRI research.^{34–36} In liquids, dissolution-DNP has already been widely integrated with
170 advanced spectroscopy and imaging experiments and has opened many new opportunities
171 including *in vivo* applications.³⁴

172

173 Other hyperpolarization schemes have also been considered and applied. For example, a brute
174 force approach would be lowering the sample temperature to millikelvin in high magnetic
175 fields.³⁷ This is, of course, not feasible for biological samples like tissues and organisms.

176 Other schemes have found success in the bioapplications; these include optical pumping with
177 noble gases (^{129}Xe) for sensing the biomolecule activities,³⁸ photochemically-induced DNP
178 (photo-CIDNP) for investigating protein folding and biomolecular interactions,³⁹ and
179 parahydrogen-induced-polarization (PHIP) for kinetic studies.⁴⁰ A common characteristic of
180 these schemes is the inclusion of chemical additives or so-called polarizing agents.

181

182 An approach to gain SNR without polarizing agents is by reducing the electronic thermal
183 noise with a cryogenically cooled rf detection coil. It requires a specific probe design,
184 denoted as cryoprobe, allowing to reduce the operating temperature of the coil to around 20 K
185 while keeping the sample at an ambient temperature. Cryoprobes have become routinely
186 employed in liquid-state NMR application to biomolecular research.⁴¹ The coil is constructed
187 with a superconducting wire, such as $\text{YBa}_2\text{Cu}_3\text{O}_{7-d}$ (YBCO), with zero resistance under 20–
188 30 K, reducing the electronic noise and can lead to an SNR gain up to 4–5 folds. The use of a
189 cold coil is of particular interest in the microcoil probe because of the increased noise level as
190 the coil size decreases; therefore, it is logical to integrate the cryoprobe technology with the
191 microcoil. Brey and his team⁴² have designed and constructed a high-resolution capable
192 cryoprobe with a 1-mm coil for liquid-state NMR spectroscopy. The probe has an active
193 sampling volume of 6.3 μL with superconducting YBCO coils cooled to about 20 K. The
194 resultant mass sensitivity (SNR per unit mass) is more than 20 times higher than a standard 5-
195 mm probe. One of the engineering challenges in cryoprobe is implanting a sharp thermal
196 gradient of a vacuum barrier between the confined space of the cold coil and the ambient
197 sample without relinquishing the filling factor. This indeed has dramatically hindered the
198 development of cryoprobes under MAS conditions. The first probe, so-called cyrocoil MAS,
199 was introduced in 2008 designed and constructed by Mizuno with his team at JEOL,⁴³ but
200 only recently, a more accessible probe was released by Bruker (BioSolids CryoProbe™) and
201 has found succussed in the biosolids.⁴⁴ The notion of 'high-resolution' spectroscopy would
202 hugely complicate the design and construction of a cyrocoil 'HR'-MAS probe. To this day,
203 only one attempt has been made by Doty and his team.⁴⁵

204

205 As these enhancement schemes require substantial instrumentation and operation costs, it is
206 of interest to explore cost-effective alternatives. One approach is optimizing the technology
207 in the rf coil efficiency, the B_1/i term in Equation 3, by using rf microcoils.

208

209 **Radiofrequency microcoil.** Electronically, the rf coil – the closest probe component to the
210 sample – functions as an inductive antenna that receives the voltage-induced rf transmitting
211 by the precession of the spin magnetization of the sample. The transverse rf magnetic field
212 efficiency, B_1/i , of an rf coil plays a role in SNR and has been discussed in detail by Hoult
213 and Richards,³² and reviewed by Webb.⁴⁶ It implies that the SNR is inversely proportional to
214 the diameter of the saddle and solenoid coil; thus, the gain in sensitivity can be achieved by

215 miniaturizing the coil around the small sample volume. Herein, we will focus on the solenoid
216 coil. It has a cylindrical geometry by winding a long wire with multiple helical turns
217 producing an effectively strong and uniform transverse field inside the cylindrical volume.
218 The B_1/i term in Equation 3 can be expressed using Biot-Savart law,

$$219 \quad \frac{B_1}{i} = \frac{n\mu_0}{2r} \frac{1}{\sqrt{1+(l/2r)^2}} \quad (4)$$

220 where n is the number of helical turns, r and l are the radius and length of the solenoid coil,
221 and μ_0 is the permeability of the vacuum. Evidently, with a fixed $l/2r$ factor, B_1/i increases
222 with the inverse of the coil diameter. Minard and Wind have outlined a didactic guideline for
223 designing μ -size solenoids ($2r \leq 1$ -mm) with optimal rf performance.^{47,48} Sweedler and Webb
224 and their team have exploited the simplicity of the solenoid design and demonstrated the
225 possibility of acquiring high-quality ^1H NMR signals of nano-volume samples with coils less
226 than 1-mm in diameter.^{14,49} This result has set off a substantial interest in academic and
227 commercial research on microcoil probe technology and made significant advancements in
228 studying volume/mass limited samples. There are several review literature on microcoil NMR
229 spectroscopy.^{46,50-56} Indeed, the capability of analyzing small volumes makes it a natural
230 choice for coupling with chemical microseparation techniques⁵⁷ and with microfluidics. The
231 latter includes an innovative hand-held NMR unit, referred to as diagnostic magnetic
232 resonance (DMR), developed by Weissleder and his team.⁵⁸ It integrates a microcoil with a
233 microfluidic system to rapidly screen biospecimens such as bacteria and tumor cells by
234 discriminating their transverse relaxation rates. This technique has recently advanced in
235 conjunction with DNP, denoted as hyperpolarized micromagnetic resonance spectrometer
236 (HMRS).⁵⁹ It permits an ultra-high sensitivity detection for a real-time NMR analysis of the
237 metabolic flux in living cells. The possibility of noninvasive monitoring with HMRS of
238 tumor progression and treatment efficacy had been proposed to be tested in clinical trials.⁵⁹ In
239 the commercial sectors, they have adapted a balance between sensitivity and sampling
240 volume for a routine-based biomolecular study. For example, Bruker has incorporated a 1-
241 mm diameter coil with an active sampling volume of about 2.5 μL .⁶⁰

242
243 Although the microcoil technology is well adapted in the NMR studies of biofluids and has
244 continued to advance in novel applications, it is entirely different for samples under MAS (or
245 microcoil MAS). This is due to the engineering challenges of implementing a microcoil
246 inside a MAS stator that can endure a rapid sample spinning. The early developments (with
247 coil diameter ≤ 1) are explicitly targeted on solid-state experiments. This is because of the

248 considerable advantage of fast sample spinning experiments with small rotors and the
249 strong B_1 field strength from the small coil. The first successful experiment was carried out in
250 2006 by Kentgens and his team using a so-called piggyback design.¹⁷ To overcome the
251 challenge of implementing a microcoil, a standard 4-mm stator was used to spin a standard
252 rotor piggybacking an μ -size sampling capillary centered inside a microcoil coil cavity. This
253 clever design permits a stable spinning of the sampling capillary driven by the conventional
254 stator while enhancing the signal with a microcoil. The results had demonstrated the
255 possibility of acquiring isotropic spectra on μ g of solids and the B_1 efficiency of producing a
256 strong decoupling B_1 field for line narrowing experiments.

257

258 In 2012, JEOL introduced the first commercial microcoil MAS probe with a 0.75-mm
259 stator,¹⁹ and shortly followed by Bruker countering with a 0.7-mm probe.²⁰ These probes are
260 capable of sample spinning as high as 110 kHz, offering the possibility of suppressing the
261 large nuclear spin magnetizations ascribed from the spin-interactions to yield narrow NMR
262 lines for characterizing solids. Hence, these microcoil MAS probes are also known as
263 ultrafast MAS. Recently, a research team has introduced a 0.5-mm MAS probe that offers a
264 groundbreaking sample spinning at 170 kHz.²¹ These commercial ultrafast probes have now
265 widened the NMR applications on biosolids, including ^1H NMR,²²⁻³⁰ an area that was first
266 considered impossible because of the intrinsically broad ^1H signals in biosolids.

267

268 The extensive developmental works in the ultrafast MAS probe have been primarily focused
269 on increasing the sample spinning and the rf field efficiency and reducing the sample heating
270 ascribed to the rotor friction and rf. There is little attention on the line broadening caused by
271 the static probe materials, which sample rotation cannot suppress. Consequently, the optimal
272 line resolution from these probes is insufficient for ^1H metabolic investigation on samples
273 like tissue biopsy. Most ultrafast MAS probes offer 0.01–0.02 ppm linewidth at best and
274 often with a distorted lineshape that field shimming cannot correct.

275

276 **Line resolution.** To comprehend the source of the line-width and -shape of an NMR
277 resonance, one must understand the origins of the effective field \mathbf{B} experienced by the sample
278 (Equation 1), which depends on the susceptibility factor χ arising from the local dipole
279 magnetic moment m . For example, when a metabolite is immersed in an isotropic solution, its
280 susceptibility induces an additional field upon the metabolite and results in a resonance shift.

281 However, metabolites in a complex biological medium are usually distributed in different
 282 cellular compartments and morphologies, each with a different susceptibility field.
 283 Consequently, this creates a heterogenous field for the metabolic protons to resonate at a
 284 series of varying resonance shifts resulting in line broadening. In NMR, this is known as
 285 magnetic susceptibility broadening.

286

287 Several convenient approaches to numerically determining the susceptibility fields inside and
 288 outside volumes of different shapes.^{61,62} One approach divides the local medium into finite
 289 volume elements and the sum of all the different magnetic dipole field contributions. In a
 290 simple case of small $\chi \leq 1$ (as in most samples), the dipole field B_z can be expressed by an
 291 angular dependence function,

$$292 \quad B_z(\theta, r) = \frac{\mu_0 m (3 \cos^2 \theta - 1)}{r^3} \quad (5)$$

293 where m is the individual magnetic moment, θ is the angle of the dipole vector to B_0 , and r is
 294 the distance apart. Note that B_z determines the resonance shift, which leads to line
 295 broadening, in the nuclear Larmor frequency induced by m , but it vanishes in the far distant
 296 field or along the surface of a magic-angle cone, $\cos^{-1}(1/\sqrt{3}) = 54.74^\circ$ to the external field.
 297 This angle is significant for line-narrowing, which will be covered in a later section. A simple
 298 numerical estimation of the B_z field can be deduced using an ideal spherical model (Figure
 299 1a), where a magnetized sphere with a radius of a and a permeability $\mu_{\text{sphere}} = 1 + \chi_{\text{sphere}}$
 300 submerged in a sample with $\mu_{\text{sample}} = 1 + \chi_{\text{sample}}$. The different dipole fields dispersed outside
 301 the magnetized sphere can be simplified as,

$$302 \quad B_z(\theta, r) \approx -\frac{\Delta\chi a^3}{3 r^3} B_0 (3 \cos^2 \theta - 1) \quad (6)$$

303 where $\Delta\chi = \chi_{\text{sample}} - \chi_{\text{sphere}}$. For example, the resonance frequency of the water molecules (χ
 304 $= -9 \times 10^{-6}$ SI unit, the negative value indicates diamagnetism) in a water bath containing a
 305 spherical air bubble ($\chi \approx 0$) can shift as high as 6 ppm when $r = a$. This shift decreases as r
 306 increases, say to $3a$; the shift is about 1.3 ppm, in-line with a typical linewidth of the water
 307 resonance. A similar effect can be found in the metabolite protons residing around the
 308 different cellular compartments, and each corresponds to a different magnetized sphere.

309

310 Another source of the B_z field is from the nearest probe components, which can be significant
 311 in the μ -size probe, where the entire sample volume is only a submillimeter apart from the
 312 magnetized materials such as the microcoil. For example, Webb and Grant⁶³ have shown a

313 threefold increase in the linewidth when a nano-volume sample is centered inside the
314 solenoid with only 50- μm apart from the microcoil.

315

316 There are several approaches to suppress B_z ascribed to the coil. These include the use of a
317 zero-susceptibility wire. It is a copper-alloy with a positive susceptibility metal, such as
318 aluminum ($\chi = +20.9 \times 10^{-6}$)⁶⁴ or rhodium ($\chi = +168 \times 10^{-6}$),⁶⁵ to cancel the negative
319 susceptibility contribution of the copper. However, the cost of manufacturing zero-
320 susceptibility wire is high because of the difficulty in producing precise and reproducible
321 doping content with small diameter wires. An academic group has proposed a laboratory
322 method of constructing a zero-susceptibility coil using a hollow copper capillary filled with a
323 specific concentration of a paramagnetic solution NiSO_4 neutralizing the negative
324 susceptibility of copper.⁶⁶

325

326 Another approach to minimize the B_z effects is to surround the microcoil with a cylindrical
327 medium of matching susceptibility creating an infinite long uniform field around the sample
328 to facilitate the field shimming. The medium can be a perfluorinated fluid such as FC-43 ($\chi =$
329 -8.8×10^{-6}),¹⁴ or an epoxy solid doped with a specific content of positive susceptibility
330 paramagnetic ions.^{67,68} The simplicity of these methods has accelerated the academic research
331 on microcoil in liquid-state NMR for producing high-resolution spectral data.

332

333 ***Magic-angle spinning.*** As stated in Equation 5, the angular dependence B_z field vanishes
334 when the dipole vector from the magnetic moment aligns with an axis at an angular distance
335 of 54.74° to the external field. The angle is known as the magic-angle. This, in turn, suggests
336 that spinning a sample at a magic-angle would eliminate the B_z field, suppressing the
337 susceptibility broadening. This was first demonstrated in the early work of Garroway⁶⁹ with
338 liquid samples and later discussed by Barbara⁷⁰ in detail of the demagnetization fields inside
339 the small volume cylindrical samples (40 μL) at magic-angle, so-called the magic-angle
340 cylinder. This essentially marked the beginning of the high-resolution standard magic-angle
341 spinning technique, widely known as 'HR'-MAS. An extensive description of the theoretical
342 and hardware of HR-MAS NMR can be found in ref 71 and 72. As illustrated in Figure 1b,
343 when the sample rotates along the axis of magic-angle θ_{MA} , the angle δ and the azimuth angle
344 ϕ become a time-dependent component given as:

345
$$\cos\delta(t) = \cos\beta\cos\theta_{MA} + \sin\beta\sin\theta_{MA}\cos(\phi(t)) \quad (7)$$

346 where β is the angular separation between the sample rotation axis and the directional dipole
 347 D_r , and ϕ is the azimuth angle on the plane perpendicular to the rotation axis. Integrating the
 348 above Equation 7 with 5, the resultant B_z field of a rotating sample becomes

$$349 \quad B_z = \frac{\mu_0 m}{2r^3} \left\{ \begin{array}{l} (3\cos^2\beta - 1)(3\cos^2\theta_{MA} - 1) + \\ 3\sin 2\beta \sin 2\theta_{MA} \cos(\omega_r t + \phi) + \\ 3\sin^2\beta \sin^2\theta_{MA} \cos(2(\omega_r t + \phi)) \end{array} \right\} \quad (8)$$

350 where ω_r is the sample rotation frequency. When $\theta_{MA} = 54.74^\circ$, the first term vanishes,
 351 leaving the second and third terms as the spinning-sideband signals that appear at multiple
 352 amplitude periods depending on the static linewidth of $\omega_r = 0$. Subsequently, MAS
 353 eliminates the susceptibility broadening B_z at rapid spinning and results in isotropic
 354 resonances at chemical shifts depending on the induced field driven by the chemical
 355 shielding. MAS also eliminates – partially in some cases – other local susceptibility fields
 356 ascribe from the spin interactions, such as spin-spin dipole, anisotropic chemical shift, and
 357 quadrupole. These interactions can contribute up to kHz–MHz in linewidth for solids.
 358 Therefore, fast sample rotation is imperative to characterize the local spin environments in
 359 solid materials.

360
 361 In the context of 'HR'-MAS, it is essential to note that MAS does not eliminate the
 362 susceptibility B_z fields ascribed from the probe materials outside the rotating sample. For a
 363 standard solid-state 4-mm MAS probe, the coil and stator (the closest components to the
 364 sample) are about 1–2 mm apart from the sample located at the wall of the rotor. This
 365 contributes to some degree of unsuppressed line broadening with the MAS. This is evident by
 366 the skew baseline for each resonance acquired from a conventional 4-mm MAS probe (Figure
 367 2). For microcoil MAS, the entire sample volume is merely a few hundredth microns apart
 368 from the coil, which renders a significant B_z field and gives substantial broadening. An
 369 example has been illustrated by an early attempt to profile a 150 μg muscle tissue using a
 370 sub-microcoil 1.6-mm MAS at 21 T.⁷³ Despite the excellent sensitivity, it is obtained at the
 371 cost of susceptibility broadening from the probe materials and obscured the J-splitting for
 372 metabolic identifications. As a result, only the lipid signals could be confidently identified.
 373 Unquestionably, the first of many challenges in the microcoil MAS development toward
 374 metabolomic applications is to achieve sufficient high spectral resolution without sacrificing
 375 the sensitivity B_1/i .

376

377 **Toward high-resolution standard microcoil MAS**

378 Since the first demonstration of high-resolution standard MAS on liquid samples,^{69,70}
 379 academic and commercial groups have invested extensive resources in developing HR-MAS
 380 probes to advance its application with high data reproducibility, user feasibility, and
 381 accessibility. As a result, HR-MAS has become the principal technique in *ex vivo* NMR
 382 studies of specimens like pathological biopsies.¹⁻⁷ On the contrary, the development of high-
 383 resolution capable microcoil MAS is scarce. The first attempt on the submilligram sample
 384 was with a bovine tissue (~300µg) using a spinning microcoil in 2007.¹⁸ But, it was not until
 385 2012 that an evaluation was made on profiling the metabolome on human tissue (<500 µg).³¹
 386 The results demonstrated a gain in sensitivity *via* the microcoil, but the ¹H line resolution was
 387 insufficient for metabolic profiling. Regardless, the results rendered a realization of an NMR
 388 application on µg-scale tissues with microcoil MAS.

389
 390 The technological issues and challenges for developing a microcoil MAS probe with a 'high-
 391 resolution' capability are different than those for a microcoil (or ultrafast) MAS probe. While
 392 faster sample spinning, stronger rf field, and reducing the frictional and electrical heating are
 393 the prime interests in the ultrafast probe developments, the high-resolution probe focuses on
 394 the high spectral resolution (down to ppb) capability with good feasibility and data
 395 reproductivity and reliability. As expected, the complication in the microcoil probe
 396 development is the complexity of handling the small size materials. A slight deviation or
 397 error from the ideal condition – whether in the probe materials, microcoil, or NMR sample
 398 preparation – will magnify its effect on the NMR performance and, therefore, on the spectral
 399 resolution. To this day, the development of high-resolution standard microcoil MAS is still in
 400 its infancy, and only proof-of-concept studies have been reported (Table 1).

401

402 Table 1. A chronological list of the significant developments that have led to 'HR' standard
 403 microcoil MAS NMR

Year	Microcoil condition (active coil volume) ^a	Highlight	Result ^b
2006 ^[17]	Static (vary; sample volume down to 10 nL)	Introduced the first microcoil MAS probe: piggyback design	<ul style="list-style-type: none"> –A µg sample capillary is piggybacked on a standard rotor –Enhanced B_1/i <i>via</i> the µcoil –Demonstrated the possibility of µg sample analysis
2007 ^[18]	Spinning (vary <900 nL)	Introduced an inductive spinning resonator: MACS	<ul style="list-style-type: none"> –An inductive resonator is placed inside a standard MAS rotor –Enhanced B_1/i <i>via</i> the inductive resonator; a mass-sensitivity of 46 mg⁻¹ is found for 750-µm MACS

2012 ^[31]	Spinning (570 nL)	Evaluated the ¹ H profiling with μ g biopsy using MACS	<ul style="list-style-type: none"> –Insufficient ¹H line resolution for metabolic analysis –Carried out on an 870-μm diameter resonator with <500 μg tissue –A 17-fold mass sensitivity enhancement compared to HR-MAS –Insufficient ¹H resolution (0.01 ppm) –Demonstrated the high versatility –Difficult in coil fabrication with no reproductivity
2012 ^[75]	Spinning (500 nL)	Introduced an automated fabrication of MACS: on-chip MACS	<ul style="list-style-type: none"> –Carried out on a 1000-μm diameter resonator –Eased the fabrication with a robotic wire-binding technology; ~100 resonators per production –Poor line resolution (1 ppm) ascribed to the large susceptibility gradient from the gold wire solenoid –Demonstrated the possibility of mass-manufactured resonators
2013 ^[73]	Static (19300 nL)	Attempt on ¹ H profiling of μ g-tissue using a standard MAS probe	<ul style="list-style-type: none"> –Carried out on a 1.6-mm MAS with a 150 μg tissue at 21 T –Good sensitivity but with poor line resolution due to the strong magnetic susceptibility gradients from the stator and coil
2013 ^[76]	Spinning (200 nL)	Refined the MACS design (First 'HR'-capable microcoil MAS)	<ul style="list-style-type: none"> –Carried out on a 550-μm diameter resonator –Offered excellent ¹H resolution (0.004 ppm) while retaining the sensitivity
2014 ^[77]	Spinning (vary: 100–420 nL)	A demonstrative metabolomic study on submillimeter organisms using HR-MACS (First metabolomic NMR study with nL volume under MAS)	<ul style="list-style-type: none"> –First metabolomic NMR study with microcoil MAS –The model study was carried out with an 840-μm diameter resonator with samples of <100 <i>C. elegans</i> individual –Introduced the use of a single fixed resonator with a sample-exchange capillary for easing the sample preparation –Successfully carried out the ¹H metabolic profiling and discrimination analysis of <i>C. elegans</i> –Limited to small scale study due to coil fragility –Single worm detection was carried out with a 400-μm resonator
2015 ^[78]	Static (<1500 nL)	Introduced a 'high-resolution' capable 1-mm microcoil MAS probe: HR- μ MAS	<ul style="list-style-type: none"> –Modified from an ultrafast 1-mm MAS probe by replacing with a specific design stator –Despite the new stator, strong rf shimming fields are needed to acquire a good line resolution (0.002 ppm),

2018 ^[79]	Static (<1500 nL)	–A demonstrative study on μg food tissues using HR- μMAS	<p>suggesting the presence of magnetic susceptibility gradients</p> <p>–Demonstrated the feasibility of 2D $^1\text{H}\{^{13}\text{C}\}$ HMQC experiment</p> <p>–First metabolomic NMR study with HR-μMAS with a model study of $n>30$ with $\sim 300 \mu\text{g}$ tissue</p> <p>–Demonstrated a reliable metabolomic study with multivariate analysis for localizing the metabolic variances in different anatomical tissues</p>
2019 ^[80]	Spinning (500 nL)	–Refined the on-chip MACS (Voxalytic TM MACS insert)	–Replaced the original gold wire with copper, resulting in a significant improvement in line-resolution (from 1 to 0.01 ppm); but still inferior to HR-MACS and HR- μMAS
2021 ^[81]	Static (<1500 nL)	–A demonstrative study on μg rodent tissues using HR- μMAS with a model study of $n>100$ (First metabolomic study on μg tissue biopsy)	<p>–Spatial metabolic mapping on tumoral brain slices</p> <p>–The large-scale study with $n>100$ of $\sim 300 \mu\text{g}$ tissue illustrated the capability of acquiring good reliability and reproducibility spectral data</p> <p>–Delimited the tumor lesion using multivariate analysis</p>

404 (a) Estimated value from the solenoidal dimension; (b) line resolution were deduced from a sucrose
405 resonance

406
407 The first high-resolution capable microcoil MAS probe⁷⁶ was a modification of an early
408 success ultrafast microcoil probe, which used an inductively coupled spinning μ -resonator,
409 so-called magic-angle coil spinning (MACS).¹⁸ It is essentially a self-resonant solenoid coil
410 wrapped closely around a nano-volume capillary and is secured inside a standard large MAS
411 rotor. The setup renders an ensemble spinning to suppress the susceptibility B_z fields from the
412 rotating microcoil and the sample and gain sensitivity *via* the inductively coupled microcoil.
413 One advantage of MACS is the resonator's dimensions versatility. It enables tailoring the
414 active and sample volume to accommodate the optimum filling factor. However, the spinning
415 of a coil under a magnetic field inherently generates an eddy current within the conductive
416 wire, producing heat *via* the dissipated power. The power can be estimated by modeling a
417 single rotating loop of a conductive wire,⁸²

$$418 \quad P = \omega_r^2 B^2 \left(\frac{r h^3 w}{\rho} \right) \quad (9)$$

419 It depends on various factors: the effective magnetic field (B), the rotating frequency (ω_r),
420 the loop radius (r), and the wire cross-section (w), and its resistivity (ρ). Any increase of
421 these factors would lead to a rise in power and thus increase sample heating. This was evident
422 with a microcoil constructed with a 62- μm cross-section of a Cu wire, spinning at 6000 Hz

423 inside an 11.7 T magnet;⁷⁶ it heated the sample by 37 °C. In contrast, the heating decreased to
424 1.6 °C with a thinner 30- μ m Cu wire; and it further reduced to 0.02 °C with a reduced
425 spinning frequency to 500 Hz. Indeed sample heating is of concern, not only to the integrity
426 of the specimens, but it generates a temperature gradient throughout the sample volume and
427 subsequently gives rise to line broadening caused by a series of frequency shifts in the
428 resonance.⁸³ The consequences can be severe for a water (HDO) sample since the ¹H
429 chemical shift is temperature-dependence -11.9 ppb/°C.⁸⁴ Figure 3 shows a dramatic loss of
430 the sucrose anomeric proton line resolution as the spinning frequency increases. This is
431 mainly attributed to the heating originating from the eddy current arising in the spinning Cu
432 wire. For example, at a routine 5000 Hz spinning, it generates about 2 °C, efficiently
433 concealing a J-splitting of 4 Hz. In contrast, a clear doublet is well resolved with low
434 spinning (<500 Hz), but it gives rise to a dense spinning-sideband pattern in the spectrum
435 (Equation 8), and requires suppression pulse experiments to disclose the isotropic resonances.
436 The refined MACS design – thin wire coil with slow-spinning – to achieve high-resolution
437 capable microcoil MAS is referred to as HR-MACS.

438

439 HR-MACS had shown an impressive spectral quality for profiling the metabolome with only
440 12-individuals of the submillimeter nematodes *Caenorhabditis elegans* (Figure 4).⁷⁷ The
441 spectral similarity to the large volume HR-MAS with more than 1000 individuals is
442 unprecedented. It rendered the identification of a total of 31 metabolites, representing the
443 most NMR-identified metabolites in a MAS spectrum of a nano-volume biological sample.
444 The sensitivity enhancement with HR-MACS is 5-fold ($B_1^{HRMACS} / B_1^{HRMAS}$) higher than that
445 with HR-MAS. In other words, it would require an acquisition period of 25 \times longer for HR-
446 MAS to obtain the same level of SNR as for HR-MACS with 12-individual worms. Pushing
447 the sensitivity limit, HR-MACS identified a few metabolite signals from a single
448 submillimeter worm at 18.8 T. However, the resultant spectral resolution was poor due to a
449 field drift over the 10-h acquisition. Regardless, the result offered a glimpse of the possibility
450 for a MAS study of a single tiny organism. This study also illustrated the beneficial factor of
451 analyzing a few numbers of the organism. To avoid crowding in the cultivation progress,
452 about 100 (or fewer) individuals are cultivated in a single culture plate; this would require 10
453 plates to cultivate 1000 individuals for a single NMR sample for HR-MAS. As a result, a
454 single model study with HR-MAS would require a demanding cultivation procedure.⁸⁵ On the
455 other hand, with the possibility of analyzing a few individuals, one plate would allow

456 multiple NMR samples, dramatically reducing the cultivation procedures. This was
457 demonstrated by a discrimination analysis (OPLS-DA) of two finite *C. elegans* groups (wild-
458 type vs *slcf-1*) with <100 individuals.⁷⁷

459

460 One issue with HR-MACS is the fabrication of the tiny and fragile LC circuitry of the μ -
461 resonator. It demands high-precision procedures. The current effective method is manually
462 winding, which is a labor-intensive process with zero reproducibility. To ensure the spectral
463 reproducibility for multivariate analysis, our team introduced the use of a single μ -resonator
464 permanently fixed inside a MAS rotor and permitting a sample screening with a smaller
465 diameter sample capillary. Two metabolomic studies with multivariate statistics have been
466 performed with this setup,^{77,86} illustrating the potential in metabolomic studies. However, the
467 constant severe spinning stress exerted onto the resonator is limited to small model studies.

468

469 Several reports have acknowledged the difficulty in assembling and reproducing the μ -
470 resonators and have attempted to apply different manufacturing approaches to ease the
471 fabrications. Korvink and his team exploited the automation of a robotic wire-bonding
472 technology to wind an μ -solenoid and bind to a capacitor chip. They denoted the resonator as
473 on-chip MACS.⁷⁵ The fabrication process is carried out entirely by a robotic wire-bonding
474 apparatus, enabling to produce 100 on-chip MACS resonators in a single procedure. However,
475 the reported NMR linewidth was highly inadequate, with about 1 ppm. This was due to the
476 significant susceptibility mismatched from the gold wire ($\chi = -34 \times 10^{-6}$) used in the wire-
477 bonding. Later, a significant improvement was made on the line-resolution, from 1 ppm to
478 about 0.01 ppm, by replacing the gold wire with copper,⁸⁶ and thereafter, it was
479 commercialized as VoxalyticTM MACS insert. Another MACS design, the so-called
480 monolithic MACS, was later introduced.⁸⁷ The design is based on a planar transmission-line
481 resonator, which is self-resonant and does not require lumped-element capacitors, permitting
482 the use of 2D MEMS printing technology. Although the manufacturing was less efficient than
483 the wire-bonding, it offered a line resolution of about 0.1 ppm. Regardless, these studies
484 demonstrated the possibility of mass-production of μ -resonators for MACS. It is now a
485 matter of minimizing the susceptibility mismatched with the samples to improve line
486 resolution.

487

488 In 2015, a new high-resolution standard microcoil probe was introduced,⁷⁸ denoted as HR-
 489 μ MAS. Unlike the spinning microcoil in HR-MACS, the new probe consists of a solenoid
 490 with 1-mm in diameter incorporated inside the MAS stator with a 490 nL sampling volume.
 491 A few probe components near the sampling volume were of significant concern in the probe
 492 design to retain the high-resolution spectra. For example, to reduce the induced B_z fields, the
 493 microcoil is wound with a zero-susceptibility wire (Cu/Al), the air-bearings are made of a
 494 susceptibility-matched Vespel ($\chi = -9.2 \times 10^{-6}$), similarly the entire stator structure is made
 495 with Kel-F ($\chi = -11.6 \times 10^{-6}$). As shown in Figure 5, these modifications have significantly
 496 improved the linewidth at 50% height (FWHM), but it also exhibits a significant skew line at
 497 1% height. Based on a numerical field map simulation, the skew line is ascribed from the two
 498 Vespel air-bearings inside the Kel-F stator generating a strong unwanted quadratic field to the
 499 sample volume.⁸⁸ To overcome this, a pair of passive ferro-shim was strategically integrated
 500 inside the probehead (Figure 5) to offset the unwanted field and supplement additional shim
 501 field equivalent to the active shim field of $B_{x^2-y^2}$, which in turn produces a quadratic field at
 502 magic-angle $B_{z^2}^{MAS}$

$$503 \quad B_{z^2}^{MAS} = B_{x^2-y^2} - 2\sqrt{2}B_{zy} \quad (10)$$

504 where $B_{x^2-y^2}$ and B_{zy} are the harmonic field functions in the active NMR shims. This
 505 implementation has suppressed the unwanted perturbed fields and yielded high-resolution
 506 spectra.

507

508 In contrast to HR-MACS, HR- μ MAS is a highly versatile and robust probe with the
 509 feasibility of heteronuclear experiments. ^1H - ^{13}C HMQC experiment has been performed to
 510 profile the lactate and its brain physiological metabolism from a $\sim 300 \mu\text{g}$ brain tissue
 511 extracted from a rat model previously infused with $[3-^{13}\text{C}]$ lactate during a brain simulation.
 512 Despite the long acquisition (27 hr), a few metabolites (alanine, lactate, glutamate, glutamine,
 513 and γ -aminobutyric acid) were identified as the relevant brain lactate metabolism accordant
 514 with those found in a previous HR-MAS study.⁸⁹

515

516 The first complete study of NMR-based metabolomics with the HR- μ MAS probe was carried
 517 out on a $\sim 300 \mu\text{g}$ tissue sampling of *Allium sativum* (garlic, Figure 6).⁷⁹ This study highlights
 518 a significant ex vivo NMR application with small size sampling. It enables the investigation
 519 of small anatomical regions that would be impractical with mg-scale sampling using HR-
 520 MAS. Specifically, a total of 14 metabolic variances, including the organosulfur compound

521 cycloallicin, has been identified in the two small sub-tissue anatomies (sprout and inner
522 epidermis) inside the garlic core.

523

524 A subsequent study explored the potential of rodent tissue biopsy by spatially profiling across
525 a coronal brain slice of C6 gliomas bearing.⁸¹ Figure 7 shows that with the same acquisition
526 time, 13-min, the resultant line resolution of HR- μ MAS is *on par* with that of HR-MAS; but
527 with nearly 30-fold less in the tissue mass. In addition, the high-resolution spectra data permit
528 unambiguous identification of the NMR markers of C6 gliomas in the rat models. The small
529 sampling mass permitted a systematic metabolic mapping across the brain slice with a
530 volume voxel of $1 \times 1 \times 3 \text{ mm}^3$, rendering a delineation of the tumor lesion using a linear PLS
531 regression and a semi-quantification analysis. Thus, the study presents a unique *ex vivo* NMR
532 possibility for analyzing pathological tissue. Another aspect worth noting is that it applied a
533 high number of sampling data (nearly a 100-sample) in the model study, illustrating the
534 capability to acquire reliable and consistent data with the HR- μ MAS probe for advanced
535 multivariate statistics. Indeed, this is imperative for metabolomic studies. Note that this large
536 sampling model would not be possible with the previously discussed spinning μ -resonator
537 probe.

538

539 **Future prospects**

540 Although advancements have been made on the high-resolution standard microcoil MAS
541 probe, it has yet to be widely applied in metabolomics. This is mainly due to the lack of
542 feasibility. The main challenge lies mainly in the minuscule sample, which limits detection
543 sensitivity and sample preparation. Further developments are necessary to continue striving
544 forward to a level of application *on par* with the conventional HR-MAS NMR spectroscopy.

545

546 **Technical developments.** It should be noted that the current state of the microcoil is close to
547 achieving the limit for detection sensitivity. Further improvement by orders of magnitude is
548 not expected through B_1/i . Therefore, higher sensitivity would be achieved by incorporating
549 other enhancement schemes. As discussed previously, high magnetic fields would enhance
550 the sensitivity, but at the expense of the equipment and its running cost; moreover, it would
551 also increase the susceptibility broadening ascribed to the microcoil. Increasing the signal-
552 averaging would also increase the sensitivity by a factor of $\sqrt{\text{scan}}$, but it could demand an
553 unrealistic acquisition period. Hyperpolarization techniques could be a possible route, but

554 how to incorporate the respective strategies is not apparent. Cryoprobe technology is
555 probably the most viable; the main issue is implanting a thermal barrier between the cold
556 μ coil and the ambient sample, complicated by the MAS stator. The piggyback concept¹⁷
557 could offer a possible solution where the stator is segregated from both the microcoil and the
558 sample and could ease the implementation of an isolated cold coil. Moreover, the sample is
559 no longer constrained inside a rotor, allowing an elongated sampling volume that renders a
560 long ellipsoid field to facilitate the field shimming. The segregation of the sample from the
561 stator could also offer a more feasible solution to the NMR-sample preparation.

562
563 Sample preparation is a critical component in all bioanalytical methods. Standardized
564 protocols are necessary and have been well-established in most *ex vivo* NMR-based
565 applications.⁹⁰⁻⁹² This is not the case for microcoil MAS. Sample preparation for the NMR
566 analysis may seem trivial at first glance, but it is, in fact, a strenuous task for handling
567 specimens at a minuscule level. One must consider the entire procedure clean and quick for
568 preserving the sample integrity. The necessity of using a μ -size sample-holder requires a
569 filling procedure that can be strenuous. The current most efficient methods are simply using a
570 micro-pipette for the solution and sampling punch for tissue with a centrifuge,⁹³ but these
571 procedures are slow and demanding. Acknowledging the difficulty, the use of sampling glass
572 capillary has been proposed for the spinning resonator HR-MACS⁷⁷ and the HR- μ MAS
573 probe.⁹⁴ The advantage is that it permits the NMR-sample preparation outside the rotor,
574 simplifying the sample filling procedure. The trade-off is the loss of sensitivity with a
575 reduced coil filling factor that can be down to 50% depending on the capillary thickness.
576 Disposable Kel-F rotor (similar concept to the Bruker Kel-F bioinsert) has also been
577 considered for HR- μ MAS probe,^{78,94} but the fragility of the small plastic rotor has hindered a
578 rapid preparation. Indeed, further improvement is essential for a more feasible analysis with
579 the microcoil probe. This may require new devices, such as microfluidic, for simplifying the
580 sample filling or even an entirely new design of the microcoil probe that can facilitate the
581 entire preparation. For example, one could consider the piggyback design,¹⁷ in which the
582 sample tube is segregated from the rotor and could be manipulated or coupled with external
583 sample-filling apparatus.

584
585 **Possible applications.** The prime advantage of microcoil MAS probes is that it opens the
586 possibility of analyzing samples with limited availabilities. These can be biospecimens such

587 as tissue biopsies, cells, organisms, or aggregated biomolecules, and they can also be non-
588 biosamples such as polymeric resins. It offers the investigations of a specific phenotype in
589 isogenic specimens that would be impractical with HR-MAS because of the difficulty of
590 isolating a sufficient quantity of the target specimens. For example, in the cell biology field,
591 'HR' standard microcoil MAS would be able to explore the metabolomes in target cell-type,
592 an emerging research area in mass spectrometry that can provide a fundamental prerequisite
593 metabolic information for understanding the underlying functions and activities. Unlike cell
594 culture, isolating the target cell type directly from an animal is a daunting task; it relies on
595 specific cell sorting techniques, which often hinder the collection of large quantities
596 necessary for the profiling with HR-MAS. For example, profiling the isolated neuron and
597 astrocyte cells may help yield insights into the fundamental of intercellular metabolic
598 cooperation between neurons and astrocytes to further understand brain metabolism.⁹⁵

599

600 Another advantage is the possibility of analyzing tiny spatial tissue regions, allowing a direct
601 characterization of the metabolite in question that can be concealed in large sampling tissue
602 mass. For example, a recent *ex vivo* HR-MAS study⁹⁶ of epilepsy with a kainate-injected
603 epileptic mouse model reported an increased γ -aminobutyric acid (GABA) level in the entire
604 ipsilateral anterior region of the hippocampus, eliciting the relevancy of GABA as a
605 biomarker of the epileptogenic zone. However, the extensive sampling tissue mass (5 mg)
606 made it impossible to delineate further the epileptic zone within the region. Figure 8 shows an
607 example of benefiting the small spatial analysis (with ~ 150 μg sampling) using the HR-
608 μMAS probe; it reveals an increased level of GABA in a tiny subregion (dentate gyrus) of the
609 ipsilateral anterior hippocampus, pinpointing the epileptic location. Although the result needs
610 further confirmation, it illustrates the underlying benefit of μg sampling over the mg scale.

611

612 In health science, small sample analysis could be used as a complementary approach to
613 advanced imaging techniques for enhancing the recognition of the modified metabolic
614 patterns of the disease in tissue. The potential of firsthand monitoring of the perturbations in
615 metabolic responses of the diseases could offer a practical and immediate treatment, opening
616 the possibility in clinical NMR spectroscopy, a concept proposed by Nicholson.⁹⁷ Moreover,
617 the current gold standard in clinical tissue assessment is histopathology. When the tissue
618 mass is limited in a single biopsy, the mg tissue analysis with HR-MAS can be complicated
619 as it can compromise the histopathological assessments; but this would not be the case with

620 microcoil MAS. In addition, the excision of μg tissue from a human patient or animal could
621 reduce surgical invasiveness and permit longitudinal studies.

622

623 In agricultural science, the analysis with μg samples could provide direct insights into the
624 integration and regulation of plant metabolism: from a single seed to a grown plant. For
625 example, the germination process in a seed is complex and of great importance for
626 developing the plant seeds to form a new individual. To characterize the metabolic
627 coordination of the distinct small regions in a seed could help the development of
628 germination⁹⁸ and essentially the plant growth. This could benefit from a spatiotemporal
629 analysis on a single seed.

630

631 **Conclusion**

632 The recent developments of high-resolution standard microcoil MAS probe technology have
633 offered the opportunity for opening new applications in the *ex vivo* platform of NMR-based
634 metabolomics. However, there is no question that further improvements in NMR sensitivity
635 and sample preparation are necessary for routine analysis of μg specimens and venture into
636 clinical NMR spectroscopy. We are convinced that 'HR'- μMAS NMR analysis could become
637 an important component in the omics research, but this would take a collaborative effort
638 among the different disciplines with complementary expertise to continue advancing and
639 making high-resolution standard microcoil MAS a real possibility in metabolomics and omics
640 in general.

641

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646 (GIN, France) is also acknowledged for the preliminary unpublished results on the epilepsy
647 tissue in Figure 8.

648 **Conflicts of Interest:** The authors declare no conflict of interest.

649 **Data availability:** All of our works present in the review are available from the
650 corresponding author upon reasonable request.

651

652

653 **Figure Captions**

654 **Figure 1.** (a) Magnetic dipole field B_z at point P of the sample (μ_{sample}) region outside of a
655 magnetized sphere (μ_{sphere}), of which it has a radius of 'a' and a dipolar magnetic moment m
656 induced by the external NMR field B_0 . δ is the angle between B_0 and the dipolar vector D_r ,
657 which depends on a distance r between m and P. (b) The MAS effect, where δ becomes a
658 time-dependent component $\delta(t)$ with a magic-angle $\theta_{\text{MA}} = 54.74^\circ$, and β is the angle between
659 the direction of the rotation axis and D_r . and ϕ is the azimuth angle characterizing the
660 position of a magnetic moment m in a plane perpendicular to the rotation axis.

661
662 **Figure 2.** ^1H spectral comparison of sucrose in D_2O at 11.7 T between solid-state MAS
663 probes and high-resolution capable MAS probes. The arrow depicts the development toward
664 high spectral resolution capable without forfeiting the detection sensitivity.

665
666 **Figure 3.** (a) ^1H doublet of an anomeric proton in sucrose, illustrating the loss of line
667 resolution as the MAS frequency increases with a spinning resonator HR-MACS. (b) A
668 summary of the FWHM in (a) and its estimated heat dissipation. The employed spinning
669 resonator was constructed with a 30- μm cross-section Cu wire. Adapted with permission
670 from ref 76. Copyright 2013 American Chemical Society. Note that the temperature
671 dependence on the chemical shift of the anomeric proton has also been observed in HR-
672 MAS.⁸³

673
674 **Figure 4.** ^1H spectral comparison at 18.8 T between HR-MACS and HR-MAS of *C. elegans*.
675 Despite a drastic reduction of the worm individuals in HR-MACS, the chemical shift
676 expansion showcases the detailed similarity in the resonance profile. Adapted with
677 permission from ref 77. Copyright 2014 American Chemical Society.

678
679 **Figure 5.** (a) Improvement of the line resolution with a pair of passive ferro shims mounted
680 near the MAS stator. The ^1H spectra of sucrose in D_2O were acquired at 11.7 T spinning at
681 4000 Hz. (b) The numerical field simulation results show the 1D magnetic flux profile within
682 the active sample along the magic-angle axis ascribed by the cylindrical bearings inside the
683 stator (B_{bearing}), a pair of ferro-shims (B_{passive}), and the combined effect (B_{sum}). The red
684 highlight represents the sample region along the magic-angle axis Z' . Adapted with
685 permission from ref 88. Copyright 2019 Springer Nature.

686
687 **Figure 6.** (a) ^1H spectral comparison of the different anatomical garlic regions between HR-
688 MAS and HR- μMAS . (b) The resultant S-line plot was used to identify the metabolite
689 variances between the garlic core's two sub-regions (sprout and inner epidermis). A result
690 that could not be easily deduced by the large-volume sampling HR-MAS experiments.
691 Adapted with permission from ref 79. Copyright 2018 American Chemical Society.

692
693 **Figure 7.** (a) ^1H spectral comparison of the rat brain tissue between HR-MAS and HR- μMAS
694 NMR at 11.7 T: black-line corresponds to HR-MAS of the tumoral (C6-glioma) lesion tissue,
695 red and blue correspond to HR- μMAS of the tumoral and healthy tissue, respectively. The
696 spectral amplitudes are normalized to the tCr resonance. Based on the total SNR (1.0–4.2
697 ppm), the mass-sensitivity per unit time for those acquired with HR- μMAS is about 3-fold
698 higher than HR-MAS. The significant NMR-markers are annotated with arrows indicating the
699 increased and decreased level: red corresponds to tumoral tissue and blue healthy. (b) A PCA
700 score plot was obtained from a model study containing 90-sampling data on brain tissue, 34
701 from a single healthy brain slice, and 56 from two tumor-bearing slices. The excellent quality

702 parameters indicated the reliability of model data acquired by the HR- μ MAS probe. (c)
703 Resultant analytical maps: [left] PLS-regression map showing the delineation of the tumor
704 lesion. The level of the predicted values is coded with a color scheme: red being 1
705 corresponds to tumoral tissue and blue being 0 to healthy tissue; [right] metabolite
706 distribution map of tCho/NAA across the slice. Each color square in both maps is relevant to
707 a 1×1 sampling pixel. Adapted with permission from ref 81. Copyright 2021 John Wiley and
708 Sons.

709
710 **Figure 8.** *Ex vivo* localized ^1H HR- μ MAS spectra of the three regions (dentate gyrus (DG)
711 dispersion and cornu ammonis (CA1 & 3)) in an ipsilateral anterior of the hippocampus of a
712 kainic acid-mesiotemporal lobe epilepsy mouse model, with a sampling mass of $\sim 150 \mu\text{g}$.
713 Similar to the result in Figure 7, the mass-sensitivity per unit time for HR- μ MAS is about 3-
714 fold higher than HR-MAS. This is an unpublished preliminary result. The spectral result
715 demonstrates the beneficiary of μg -sampling with HR- μ MAS over mg -sampling with HR-
716 MAS that has concealed the spatial origin of the evaluated GABA level.

717
718

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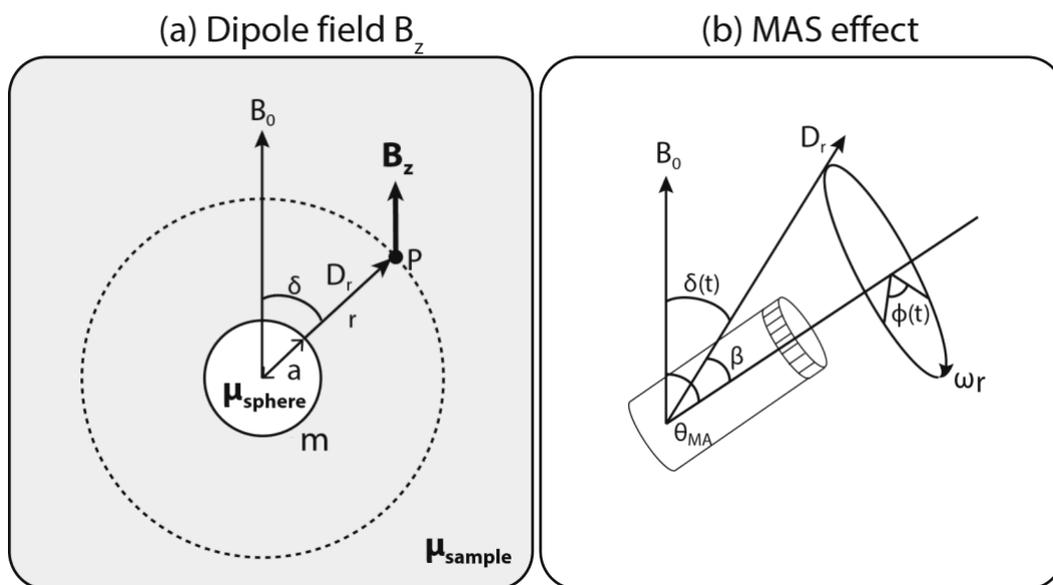
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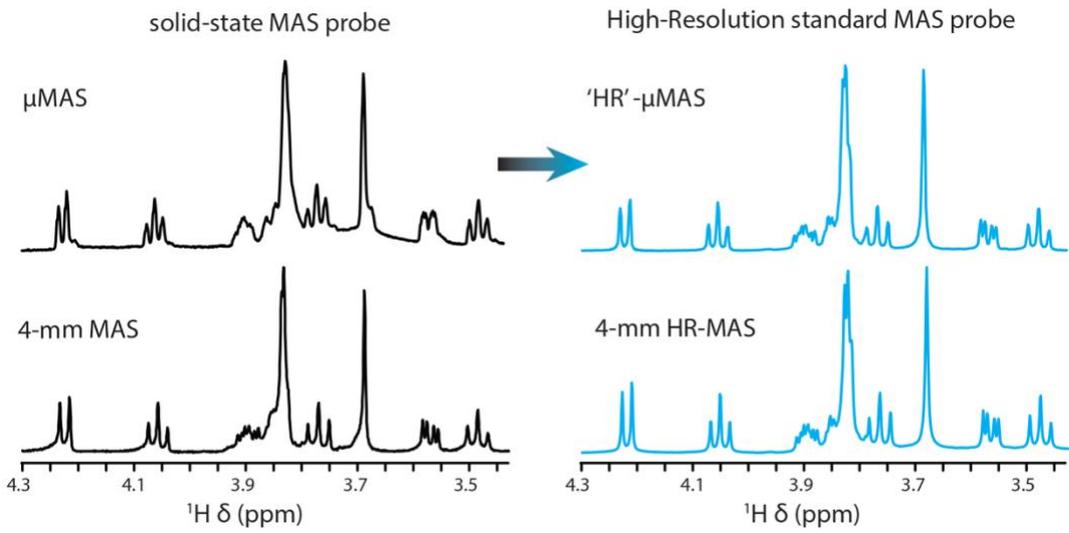
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Figure 1

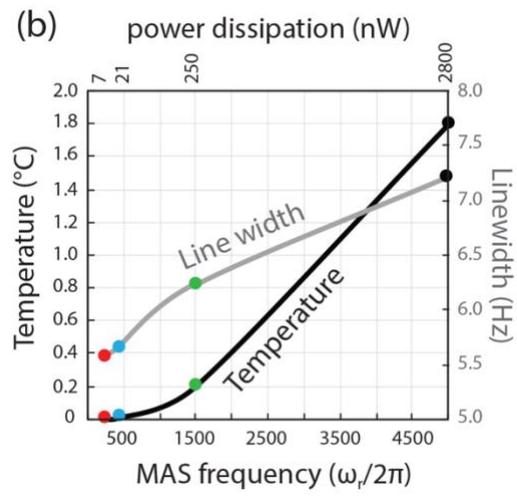
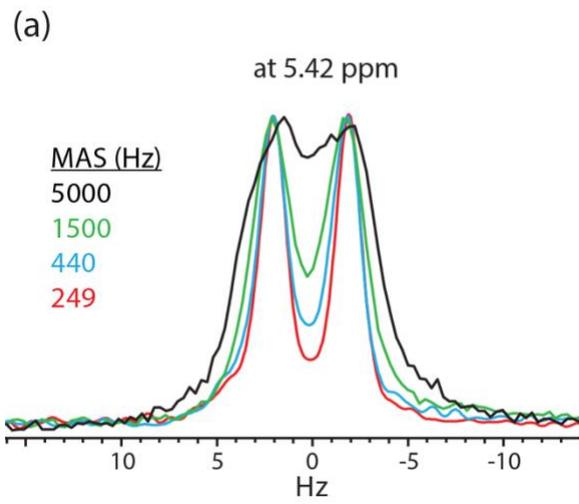
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Figure 2

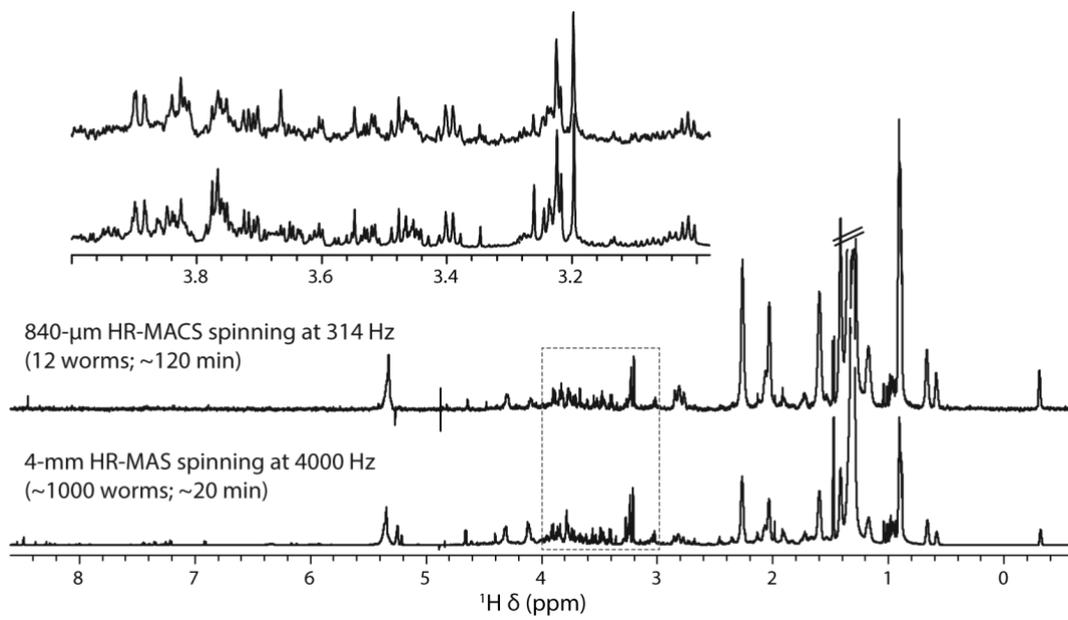
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Figure 3

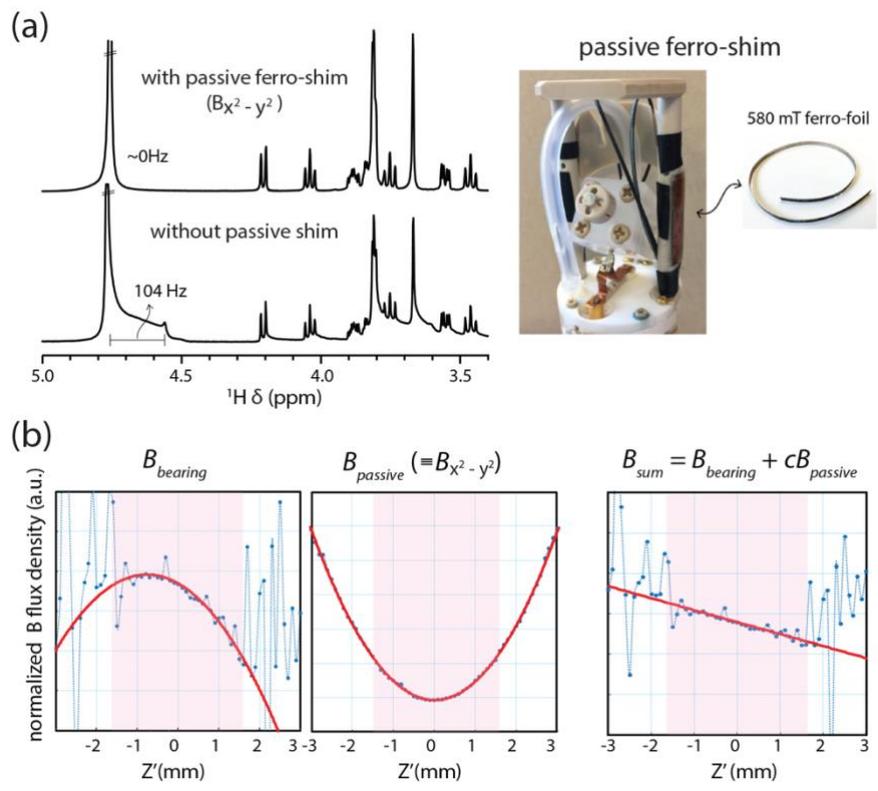
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Figure 4

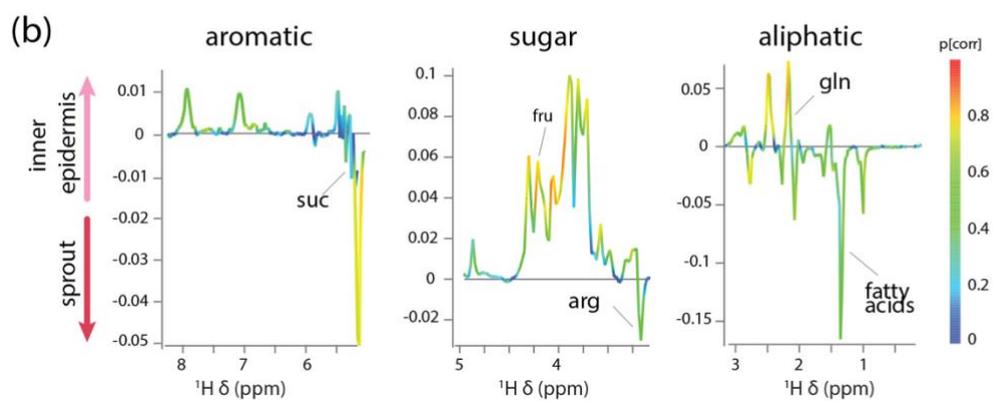
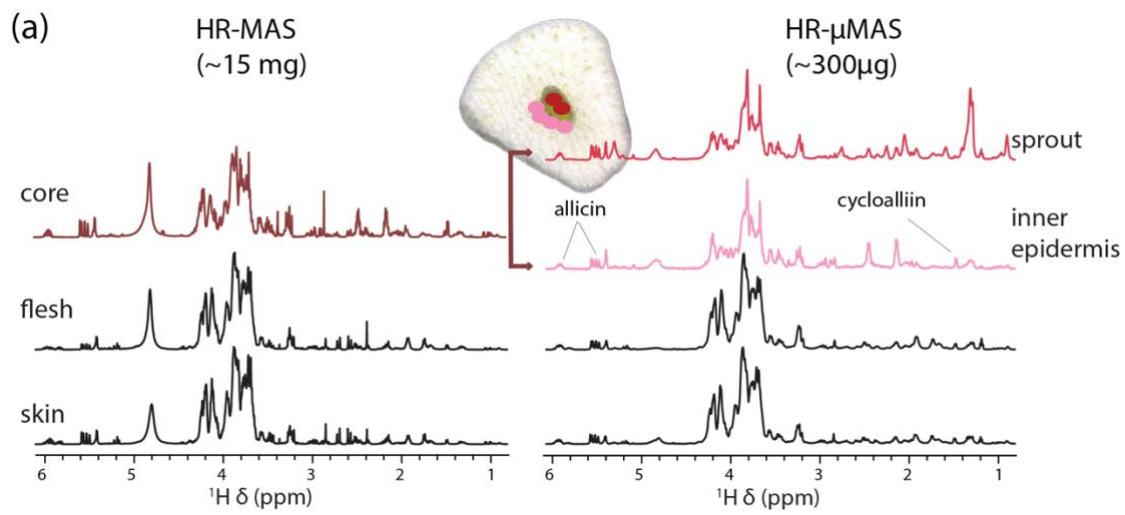
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Figure 5

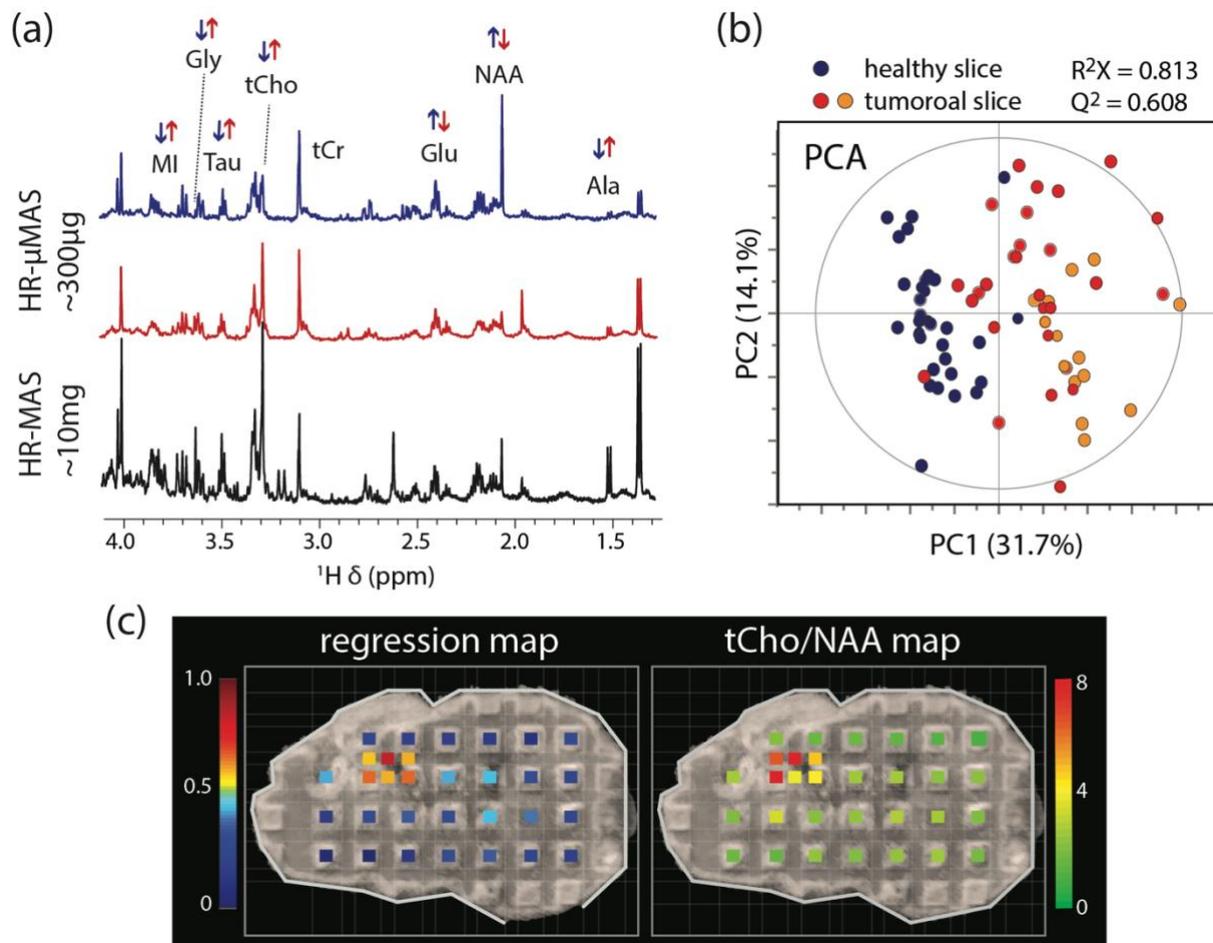
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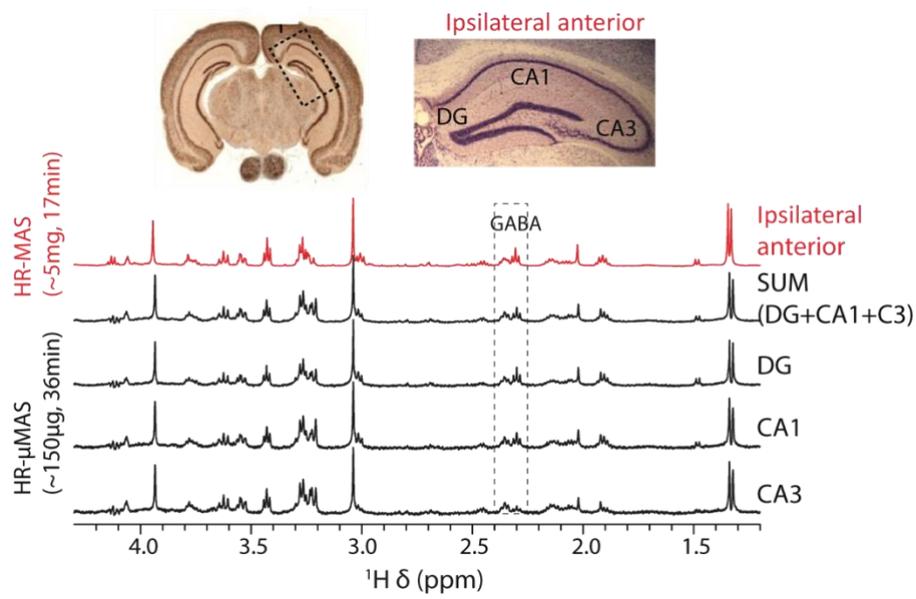
Figure 6

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Figure 7



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Figure 8