

2019 volume 29 number 4

## *Tg[LC3:GFP]* (monitoring autophagy)



The Publication of the International EPR (ESR) Society



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Please feel free to contact us with items (news, notices, technical notes, and comments) or ideas for the *EPR newsletter*.

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The cover picture illustrates aspects of the research carried out by Hassane Mchaourab, recipient of the IES Silver Medal for Biology/Medicine 2019. It shows confocal microscopy images of zebrafish lens expressing fluorescently tagged proteins.







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#### **Editorial**

Dear colleagues,

It would be perfectly understandable if actually it took you some time to stop looking at the cover of this issue illustrating some aspects of the research carried out by Hassane Mchaourab, recipient of the IES Silver Medal for Biology/Medicine 2019. The cover is really breath-taking and spellbinding. It is like looking into the mysterious black depth of the Universe with floating cosmic bodies.

Now, since you have finally turned to the contents of the newsletter, let me introduce you into what we prepared for you this time.

First things first: the results of the glorious celebration of the 75 years of EPR and 30 years of the IES are summarized in the Letter of the President by Thomas Prisner (p. 3) and the report of the IES Annual General meeting 2019 (pp. 3–5). Both present the most important activities of the IES in order to promote the development of EPR with an emphasis on EPR schools for young scientists, poster awards, IES Best Paper Award, etc. These activities are featured in the issues of our publication.

Short personal accounts presented at the Rocky Mountain conference dinner IES celebration by Gareth Eaton (EPR Instrumentation, pp. 17, 18) and Christoph Boehme (The Past, the Present and the Future of EPR Spectroscopy for Physics and Materials Sciences, pp. 19–21) are given in detail in this issue. Sure it is a must-read for everybody in the magnetic resonance community, especially for the newcomers in the field.

Messages to the young generation of magnetic resonance researchers given by Hassane Mchaourab – "Find an important question that you can uniquely address, appreciate the scientific landscape of your field, be aware of competition but most importantly work hard." (p. 6) – and Hitoshi Ohta (International Zavoisky Award 2019) – "the research a bit different after your PhD may expand your knowledge and experiences, and may give an important influence on your future" (p. 9) – are gold guidelines to those who start their career in science.

We all join in congratulating Hassane and Hitoshi on their well-deserved awards, the same as Daniella Goldfarb and Angela Gronenborn (2019 Richard R. Ernst Prize) and Klaus Möbius (Medal "To the Memory of Academician N. M. Emanuel"). And we readily join Daniella on her long and winding road to in-cell DEER with Gd(III) (pp. 10–16). Please do not miss conference reports on the International conference "Magnetic Resonance – Current State and Future Perspectives (EPR-75)" in Kazan and the XIth EFEPR conference in Bratislava. They are shifted to the forthcoming issue.

I wonder if somebody manages to guess what place is shown in the beautiful landscape drawn by Thomas Prisner illustrating his Letter of the President. It is not for the first time that Thomas shares with us his other passion featured in the article "Curve sketching and line etching" (20/1, pp. 6, 7); see also the Christmas card (20/1, p. 2), the cover of the EPR newsletter 18/1 – an etching dating back to 1990 when Thomas worked as a postdoc in the laboratory of Robert Griffin and being a collage of impressions from MIT, Charles River skyline and figures and calculations out of his notebook -, and the view of the Vallombrosa monastery he drew at the11th Chianti Workshop (17/2-3, p. 36).

Interested in another passion of our outstanding scientists?! Welcome to the relevant column in our publication started with George Feher's "Playing poker" (13/1-2, pp. 10–12)!

Laila Mosina



## Letter of the President

Dear EPR colleagues, dear IES members,

Again a year almost past! Good time to reflect.

2019 was the 75th anniversary of EPR, celebrated in Kazan in September at the EPR-75 conference. Indeed EPR is a grown up technique with a tremendous broad portfolio of applications in analytics, materials sciences, physics, chemistry, geology, structural biology and medicine – just to name a few of them! Surprisingly, there are still new method developments going on, which keeps EPR young and fascinating for fundamental science and research. This very much set also the spirit at various EPR schools, which took place this year.

IES engaged and supported the US EPR School, organized by Gary Gerfen and Stefan Stoll from Shared EPR and hosted by Sandra and Gareth Eaton in Denver this summer and the EFEPR winter school in Czech Republic, organized by Petr Neugebauer. For me it was very stimulating to talk to the young students, who were so excited about EPR and interested in learning EPR techniques and basics. I believe that this is one of the most important activities to foster EPR and to which IES can contribute!

This year was also the 30th anniversary of the International EPR (ESR) Society. This was celebrated at the Rocky Mountain Conference in Denver and at the Kazan EPR-75 meeting. Many things happened in EPR over this timespan; this was nicely highlighted in short personal accounts presented at the Rocky Mountain conference dinner IES celebration by Harold Swartz, Gareth Eaton and Christoph Boehme. Of course also the view on purpose and use of a society changed within this time! We are very happy that EFEPR hosted the Annual General Meeting of IES this year at the very nice EFEPR meeting in Bratislava in September, where we got a number of new IES members. The executive board of IES hopes very much that you all enjoy the benefits of the society, as for example the EPR



Newsletter, so beautiful edited by Laila Mosina over many years! More recently, Songi Han and Aharon Blank added a Twitter page to our Webpage. I hope that you like to see all the posts from EPR and related scientists there as well as the announcements of new publications related to EPR. Please join in with your own contributions and pictures! Additionally, we rewarded this year for the first time the IES Best Paper Award for young scientists to Daphné Lubert-Perquel. This year, the Silver Medal in Biology was awarded to Hassane Mchaourab and the Silver Medal in Physics to Robert Bittl. Lawrence Berliner and Michael Mehring were elected as Fellows of the Society.

I wish all of you relaxation (which usually we don't like so much in magnetic resonance) and a very productive and successful New Year 2020!

Looking forward to meeting you at conferences next year!

Best regards, Thomas

## **IES ANNUAL GENERAL MEETING 2019**

Minutes of the Annual General Meeting of the International EPR/ESR Society for 2019, held during the EFEPR Meeting, STU, Bratislava, Slovakia, September 4, 2019.

#### Agenda:

- 1. Introduction (T. Prisner)
- 2. Report of last year activities (T. Prisner)
- 3. Secretary's Report (A. Blank)
- 4. Treasurer's Financial Report(A. Blank)
- 5. EPR newsletter Editor's Report (L. Mosina)
- 6. Planned IES Activities in 2020 (H. Ohta)
- 7. New Possible Initiatives (H. Hirata)
- 8. Thanks

#### 1. Introduction by Prof. Thomas Prisner

Dear Colleagues,

On behalf of the IES Executive Board I wish to welcome all participants to the EFEPR Meet-

ing and the International EPR(ESR) Society, STU, Bratislava, Slovakia, September 4, 2019.

I would like to express my gratitude to the conference organizers of this meeting, especially to Michal Zalibera and Peter Rapta, and for allowing our General Meeting to take place during this Conference.

#### IES executives (2018-2020)

President: Thomas Prisner Vice President Asia Pacific: Hiroshi Hirata Vice President Americas: Songi Han Vice President Europe: Gunnar Jeschke Secretary: Aharon Blank Treasurer: Peter Qin Immediate Past President: Hitoshi Ohta EPR newsletter Editor: Laila Mosina 2. Short Report of the previous AGM at the Third Joint Conference of the Asia-Pacific EPR/ESR Society and the International EPR(ESR) Society on September 26, 2018 Brisbane, Australia by Thomas Prisner

See EPR newsletter 28/4 (2018) pp.3-5

#### Prizes Awarded in 2019

- Silver Medal in Biology/Medicine: Hassane S. Mchaourab
- Silver Medal in Physics/Materials Science: Robert Bittl
- Fellows:
- Lawrence J. Berliner; Michael Mehring

  Poster Prize Winners:
- RSC ESR Meeting Glasgow Luis Fabregas Ibanez (ETH Zürich) Benjamin Tucker (Oxford) Rocky Mountain Conference Denver

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#### Annual General Meeting 2019

Joseph E. McPeak (Denver) Elizabeth Canarie (Washington) EUROMAR/ISMAR Berlin Yury Kutin (TU Dortmund) Steven Worswick (Southampton) EPR School Denver 2019

- First EPR School in US (July 17–21, Denver)
- Initiated and co-sponsored by IES and Shared EPR
- 40 Students (200 Applications)
- 4 days with lectures, tutorials and practical courses
- 30 years of IES at Rocky Mountain Conference

During the Rocky Mountain Conference we had a special session of short presentations of the development in the last 30 years in the fields:

Physics/Materials Sciences (Christoph Boehme) Biology/Medicine (Harold Swartz) Chemistry & Instrumentation (Gareth Eaton) Moderator: (Thomas Prisner)

#### 3. Secretary's Report, Aharon Blank

The Secretary is responsible for the day-to-day operations of the Society, and ensures efficient functioning of the Society, e.g.:

1. The Secretary shall maintain all the records of the Society, shall keep the minutes of Society meetings, and be responsible for the distribution of all essential information to members.

2. Sending out invoices to the sponsors (in consultation with the Treasurer).

3. Informing members (and sponsors) of the various items of interest, e.g., announcements of conferences, workshops, publication of new issues of EPR newsletter.

4. Organization of material for awards given by the IES: medals, certificates and citations.

5. Overlooking financial status and membership of the Society (in consultation with the Treasurer).

6. Website: maintenance and upgrades, ads and positions. We are looking for interesting photos, papers, links (see next) – please send them out.

- 7. Answering any enquiries.
- 8. Organizing AGM.

9. Liaisons with the President, Treasurer, Editor of the *EPR newsletter*, and the members of the IES Executive.

#### Major tasks over the last year:

1. IES members list cleanup, accurate EPR newsletter delivery addresses

2. Handling the associate membership initiative

3. Handling Awards, fellowships and the "Best paper Award" new initiative

4. Establishing the IES twitter (with Songi Han)5. Migration to a new web site host and new web admin (still ongoing)

6. Handling poster prizes at the EUROISMAR meeting

Presentation of the IES Tweeter page and its features.

Presentation of the web site and its features.

## 4. Treasurer's Report, Tatyana Smirnova and Peter Qin (Financial Reports)

#### 2018 Financial Report (\$)

Balance January 1, 2018	38,309.59
Deposits:	
Membership	9,392.91
Sponsor contributions	21,501.40
Bruker contribution to	
printing	742.00
Misc	10.00
TOTAL deposits:	31,646.31
Expenditures:	
Internet commerce and	
merchant services, banking	838.28
Web design/maintenance	
& fees	2,623.83
Newsletter printing	6,370.00
Conference support	
(APES sponsorship)	3,000.00
Awards	2,627.15
Illinois State fee; refund; etc.	136.79
TOTAL expenditures:	15,596.05
Balance December 31, 2018	54,359.85

#### 2019 January-June Financial Report (\$)

	1 (1)
Balance January 1, 2019	54,359.85
Deposits:	
Membership	948.00
Bruker contribution to	
printing	2,226.00
Transfer from JW Fund (for	
2018 award paid from main	
account)	1,004.99
TOTAL deposits	4,178.99
Expenditures:	
Credit card fees, Internet	
commerce and merchant	
services, bank fees	370.82
Newsletter printing	1,280.00
State of Illinois fee	10.00
Editorial services in 2018	3,242.00
Website re-idesign	
(1st installation)	3,960.00
IES Poster Awards	604.99
Misc (stamps, medal engravin	ıg,
etc.)	152.60
TOTAL expenditures	9,620.41
Balance June 28, 2019	48,918.43

John Weil Fund. Established in 2010 in<br/>memory of Prof. John Weil by family,<br/>friends, and colleagues to support John<br/>Weil Young Investigator Award. (\$)Balance on 01/01/201923,492.94Distributions in 2019 (for 2018 John Weil<br/>Young Investigator Award1,004.99Balance as of 06/28/201922,487.95

We want to thank Tatyana Smirnova and Peter Qin for their excellent work as Treasurer of the Society.

#### 5. EPR newsletter Editor's Report, Laila Mosina

Since the previous Annual Meeting of the IES in 2018 in Brisbane (Australia), we published single issues 28/3 and 28/4, and a double issue 29/1-2. We hope you had a look at 28/3, 28/4 and 29/1-2 on the newsletter website and got their copies.

Now we finalized 29/3 and work on the forthcoming issue 29/4. To remind you, we present the columns of the newsletter: Columns of the EPR newsletter 29/4 (2019)

- Editorial
- IES business
- Awards
- IES Young Investigator Award Revisited
- Another Passion
- Anniversaries
- EPR newsletter Anecdotes
- In Memoriam
- Present Meets Future
- Software
- Tips and Techniques
- Notices of Meetings
- Conference Reports
- New EPR Faculty



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Peter Poliak

Alexey Popov

Jabor Rabeah

Peter Rapta

**Thomas** Prisner

Ashley Redman

Edward Reijerse

Emma Richards

Jerome Robert

Marco Rosenkranz

Mohammad Samani-

Enrico Salvadori

Olav Schiemann

Max Schmallegger

Alexander Schnegg

Simon Schumann

Matúš Šedivý

Roberta Sessoli

Mantas Simenas

Miriama Šimunková

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Michal Zalibera

Tatyana Smirnova

Antonín Sojka

Jacob Spencer

Ivan Sudakov

Ián Tarábek

Ilenia Serra

Erik Schleicher

pour

Stephan Rein

Jesus I. Martinez

Vladimir G. Malkin

- New Books and Journals

- Market Place
- Reader's Corner
- Guest of the Issue

Please feel free to submit YOUR material, dear colleagues!!!

On behalf of the Editorial Board, I thank most heartily all contributors to the EPR newsletter with special thanks going to the CEOs of the IES and editors of the columns in the EPR newsletter: John Pilbrow, Candice Klug, Wolfgang Lubitz, Stefan Stoll, Keith Earle and David Budil, Sabine Van Doorslaer, and also to Yevhen Polyhach, our web-master, and Sergei Akhmin, our Technical Editor.

I gratefully acknowledge collaboration with Associate Editors Candice Klug, Hitoshi Ohta and Sabine Van Doorslaer.

We want to thank Laila Mosina and her editorial team for their excellent work for the EPR newsletter of the Society.

#### 6. Planned IES Activities in 2020, Hitoshi Ohta

Poster Awards are to be given at the following conferences:

- Royal Society Chemistry EPR Meeting Manchester (2)

- EUROMAR Bilbao (2)

– Rocky Mountain Conference Breckenridge  $\left(4\right)$ 

- Asia-Pacific EPR Meeting Yellow Mountains, China (2)

Annual General Meeting is planned during the Rocky Mountain Conference Breckenridge. • IES Awards

- Silver Medal for Instrumentation
- Gold Medal of IES
- Fellows of IES
- John Weil Young Investigator Award
- Required:
- Nominations by active IES members
- 100–150 word citation
- Publication List + pdf of two recent publications

– CV

• EPR Publication Award (for young scientists)

- Self nomination

- First author with support letter from corresponding author/supervisor which explains the contribution of first author

#### 7. New possible initiatives, Hiroshi Hirata

Create more visibility of the Society, also for young researchers!

- Invite young scientists (YIA/Poster Prize winners) to the IES Board Meeting
- Create an interactive Communication Platform on our Webpage
- Put tutorials, special articles on our Webpage
- Create a directory of EPR groups
- Host EPR database first for Dipolar EPR Data

#### 8. Thanks

The IES thanks the following Corporate Sponsors for their contributions in 2019 Bruker BioSpin Corp JEOL RESONANCE Inc Oxford Instruments NanoScience Virginia Diodes, Inc Magnettech GmbH Rotunda Adani Systems, Inc L & M EPR Supplies Research Specialties Molecular Specialties KEYCOM Corporation Norell, Inc GMW Associates

Special thanks go to ETH Zurich for hosting the Newsletter website and the Zavoisky Physical-Technical Institute, Kazan for supporting the Newsletter, and to: All paid up members Newsletter Editor: Laila Mosina Technical Editor: Sergei Akhmin Associate Editors: Candice Klug, Hitoshi Ohta, and Sabine Van Doorslaer

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Andrea Albino

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## Interview with Professor Hassane Mchaourab on the Occasion of His IES Silver Medal for Biology/Medicine 2019



**EPR newsletter:** Dear Professor Mchaourab, on behalf of the readers of the EPR newsletter we congratulate you on your IES Silver Medal for Biology/Medicine 2019. We are most appreciative that you agreed to answer the questions of this interview. Why did you start towards your career in science?

I came to realize my passion for science at age 11 after I read a book called "the hunchbacked universe". This book was a lay introduction to the theory of relativity. I was captivated by the implications of the theory from the origin of the universe to the famous twin experiment. Although in my early childhood I was profoundly influenced by my parents' nightly discussions of philosophy and political systems, reading this book represented a transformational moment that stimulated a dream of becoming a physicist.

Who introduced you into magnetic resonance? I started my Masters degree at the American University of Beirut initially as a theoretician studying supersymmetry. For a confluence of reasons, I had decided to switch my research focus and talked to the magnetic resonance group in the department. The initial plan was for me to write the theory for an experimental project of another student. The group consisted of two physicists: Frederick Heineken and Theodore Christidis who became my official advisors. Their first assignment was to read and present a classic paper by Freed and Frankel. While initially I was dead set to return to elementary particle physics, I credit Teddy Christidis for opening my horizons to the opportunities emerging in the biological applications of magnetic resonance. Having spent a summer at the National Biomedical ESR Center in Milwaukee, he encouraged me to apply for my Ph.D. studies under the supervision of Jim Hyde.

## What part of your research is most dear to your heart and why?

Although I am passionate about my current work on protein dynamics, the dearest scientific moments to my heart were those associated with the discovery of what came to be known as multiquantum EPR. I fondly recall sleepless nights as I marveled over observing these nonlinear responses to double frequency irradiation as well as days and nights deriving analytical expressions for the EPR signals. These were years of wonder and creativity that shaped my personality as a scientist and brought me into a close connection with Jim Hyde, a connection that I still cherish.

What is your message to the young generation of magnetic resonance researchers?

EPR will continue to play an important role in understanding how macromolecules work. It is a unique technique and has a bright future. The key to success is to be system and question oriented. While I encourage young EPR spectroscopists to keep EPR front of center, they shouldn't shy away from integrating it with other techniques. Find an important question that you can uniquely address, appreciate the scientific landscape of your field, be aware of competition but most importantly work hard. Working hard is still the most direct way to success in any field of science.

#### notices of Meetings

#### The 53rd Annual International Meeting of the ESR Spectroscopy Group of the Royal Society of Chemistry RSC ESR 2020

Manchester, March 29 – April 02, 2020 Web: http://www.esr-group.org/ conferences/2020-conference-manchester

#### The International BioEPR SCHOOL-CONFERENCE 2020 (BIOEPR 2020) Novosibirsk, Russia, June 28 – July 03, 2020

Web: http://bioepr2020.ru

**16-th EUROMAR conference EUROMAR 2020** Bilbao, Spain, July 5–9, 2020 Web: https://www.euromar2020.org

#### The 61st Annual Rocky Mountain Conference on Magnetic Resonance

Colorado, The Rocky Mountain, The Copper Conference Center in Copper Mountain, July 20–24, 2020 Web: https://rockychem.com

#### APES-2020

Huangshan, China, September 19–23, 2020 Web: www.apes2020.ustc.edu.cn (starts January 2020) E-mail: meetings@ustc.edu.cn

#### MDMR 2020

Kazan, Russia, September 28 – October 02, 2020 Web: http://www.kfti.knc.ru/mdmr/2020 E-mail: mdmr.kazan@yandex.ru

#### Symposium "Free Radicals in Biology, Chemical Synthesis and Functional Bio-/Nanomaterials" at the PACIFICHEM 2020

Honolulu, Hawaii, USA, December 15–20, 2020 Web: https://pacifichem.org and https://pacifichem2020.abstractcentral.com (starts April 1, 2020) Alex I. Smirnov (USA) E-mail: alex\_smirnov@ncsu.edu phone: 1-919-513-4377 Steven Bottle (Australia) E-mail: s.bottle@qut.edu.au Naoki Yoshioka (Japan) E-mail: yoshioka@applc.keio.ac.jp

## The 2019 Richard R. Ernst Prize to Daniella Goldfarb (Weizmann Institute) and Angela Gronenborn (University of Pittsburgh)



**B**ruker Biospin Corporation, together with EUROMAR, has established the Richard R. Ernst Prize in Magnetic Resonance to honor the breakthrough contributions that our colleague and friend Richard Ernst made to

the field of magnetic resonance. Specifically, the prize was established to recognize "recent beneficial applications of Magnetic Resonance" and intends to award achievements beyond just fundamental research. From left to right: Prof. Angela Gronenborn, Prof. Lucia Banci, Chair of Richard R. Ernst Prize Selection Committee, Prof. Daniella Goldfarb and Dr. Falko Busse, President of Bruker BioSpin.

The Richard R. Ernst Prize Selection Committee has voted unanimously "To award the Richard R. Ernst Prize to Daniella Goldfarb and Angela Gronenborn for their contributions to the development of magnetic resonance methods and their applications to molecular biology. Goldfarb made pioneering contributions refining and using DEER for the structural and dynamical characterization of Gd(III) spin labeled proteins within cells. Gronenborn, an early leader in developing approaches to determine the structure of biological macromolecules, has recently integrated NMR with other structural and computational methods to elucidate structure and function of viral proteins and their assemblies".

Previous winners are Alexander Pines (UC Berkeley), Robert G. Griffin (MIT), and Claudio Luchinat (University of Florence)

# SPINSCANX

#### **Highlights:**

- Cavity Q-factor and MW power measurements
- Magnetic field modulation 10kHz-250 kHz
- Phase detection range 0-360°
- First and second harmonics detection (in phase and out of phase)
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## BENCHTOP X-BAND EPR SPECTROMETER



#### Awards



## The International Zavoisky Award 2019 to Professor Hitoshi Ohta Kobe University, Kobe, Japan

The 2019 Zavoisky Award is presented in recognition of a work dedicated to electron paramagnetic resonance and, in particular, the laureate's outstanding contributions to terahertz high-field EPR instrumentation and its applications in solid-state physics.

For details, see the forthcoming issue of the EPR newsletter

From left to right: Vice Prime-Minister of the Republic of Tatarstan Leyla Fazleeva, Prof. Hitoshi Ohta, Prof. Thomas Prisner (President of the International EPR Society and Zavoisky Awardee 2014), Prof. Kev Salikhov (Chairman of the Zavoisky Selection Committee and Zavoisky Awardee 2004), and Prof. Elena Bagryanskaya (President of the Asia-Pacific EPR Society). The ceremony took place in the City Hall of Kazan on September 26, 2019.



From left to right: Prof. Alexey Trofimov (Vice-Director of the N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences), Prof. Klaus Möbius.

The Medal "To the Memory of Academician N.M. Emanuel" was established by the Russian Academy of Sciences, the M. V. Lomonosov Moscow State University and the International Charitable Fund "Scientific Partnership" in 2007. It is annually awarded to Russian and foreign scientists and institutions to mark their significant contributions to the development of physico-chemical biology, chemical kinetics, biotechnology and biochemical physics. Among the previous winners of the Emanuel Medal Prize were, for example, E. M. Pearce (USA, 2009), A. I. Kokorin (Russia, 2011), K. M. Salikhov (Russia, 2014), I. A. Grigorev (Russia, 2015), W. E. Trommer (Germany, 2015), G. Grampp (Austria, 2016).

On April 8th, 2019, the Awarding Committee of the Emanuel Medal Prize has voted unanimously that for 2019 "Klaus Möbius (Free University Berlin, Germany) is awarded with the Medal "To the Memory of Academician 2019 Medal "To the Memory of Academician N. M. Emanuel" to Professor Klaus Möbius Free University Berlin, Germany

N.M. Emanuel" for his outstanding results in the area of chemical and biochemical physics". The other winners of the 2019 Emanuel Medal are Olga Lavrik (Russian Academy of Sciences, Novosibirsk), Vladimir Arutyunov (Russian Academy of Sciences, Moscow) and Uwe Eichhoff (Bruker Biospin Corporation, Germany).

The Presentation of Diplomas and Medals to the awardees as well as their Scientific Talks took place on October 1st, 2019 at the N. M. Emanuel Institute of Biochemical Physics RAS in Moscow.

The title of Klaus Möbius's scientific talk in Moscow on 1 October, 2019, was: "High-field EPR studies of protein/matrix hydrogen-bond interactions – potential mechanisms for Life without Water?"

## Interview with Professor Hitoshi Ohta on the Occasion of His Zavoisky Award 2019



EPR newsletter: Dear Professor Ohta, on behalf of the readers of the EPR newsletter we congratulate you on your Zavoisky Award 2019. We are most appreciative that you agreed to answer the questions of this interview. Why did you start towards your career in science?

I think my aunt Kazuko Motizuki gave a great influence on me to go into physics. She was the first woman in Japan to receive PhD in theoretical physics from the former Imperial University of Japan (Osaka University). She was also the first woman full professor at Osaka University. Therefore, she was one of pioneers as a woman scientist in Japan. When I was a child she often took me to her laboratory at Osaka University during the summer vacation. It was fun because young students at the laboratory took care of me. Then as a child I also had many chances to talk to famous professors like T. Nagamiya or N. Kawai. Prof. Nagamiya

known for the antiferromagnetic resonance theory (Nagamiya-Kubo-Yoshida, 1955) was a leader in the theoretical magnetism at that time, and he was a mentor of J. Kanamori known for "Goodenough-Kanamori rule" in magnetism, T. Moriya known for "Dzyalosinsky-Moriya interaction", and K. Motizuki. Prof. Kawai is also well known for developing the Kawai-type multianvil apparatus to generate an ultra high pressure. Therefore, it was rather natural for me to go into physics after entering the University of Tokyo. However, at the last moment I made a small difference with my aunt. I took the experimental physics, which seemed much more attractive to me.

Who introduced you into magnetic resonance? Prof. Mitsuhiro Motokawa at Kobe University introduced me into ESR. My starting point was not ESR but the far-infrared spectroscopy during my graduate course before Kobe University. In 1987 I joined the group of Prof. Mitsuhiro Motokawa at Kobe University as an Assistant Professor, where my high-frequency ESR carrier started. Prof. Motokawa started the high-frequency ESR using the pulsed magnetic field in the 1970s at Prof. Date's group, Osaka University, and opened a new Laboratory at Kobe University in 1986. The new Laboratory had a small 23 kJ (3 kV) capacitor bank in operation and my task was to start up the high-frequency ESR system using the liquid He cooled 15 T pulsed magnet and the far-infrared laser. However, we had some hard times with the unstable laser. Things changed a lot when new backward wave oscillators (BWO, called carcinotron produced by Thomson) covering the 300 and 350 GHz bands were installed. We started to get many interesting results on

quantum spin systems. Ten years later Prof. Motokawa moved to Tohoku University but I remained at Kobe Univesity and continued the developments of THz ESR with my new colleagues to what you know today.

What part of your research is most dear to your heart and why?

As the early development of high-frequency ESR using the pulsed magnetic field has started in the 1970s at Osaka University and continued at Kobe University, I think my real contribution to the THz ESR is the extension to the multi-extreme THz ESR. First, we are not the only group to apply the high pressure to ESR but we really extended it to THz ESR, and showed that it is useful to study exotic phases in the magnetic systems under high pressure up to 2.5 GPa. Secondly, the similar situation is true for the mechanically detected ESR. It was first demonstrated by Rugar et al. at low frequency using the micro-cantilever. However, we have shown that it can be extended to THz ESR and highly sensitive THz ESR using the micro-membrane is now possible for the dilute spin systems including the metal proteins.

What is your message to the young generation of magnetic resonance researchers?

As mentioned above, my starting point was not ESR but the far-infrared spectroscopy during my graduate course. However, the experiences of far-infrared spectroscopy experiments, for instance, about detectors and light sources, helped me a lot when I started the developments of THz ESR at Kobe University. Therefore, I feel that doing the research a bit different after your PhD may expand your knowledge and experiences, and may give an important influence on your future.



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# The long and winding road to in-cell DEER with Gd(III)

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#### Introduction

The cellular environment is complex and differs considerably in terms of viscosity, confinement and composition from in-vitro conditions, under which proteins' structure and dynamics are commonly studied. These different conditions can affect conformational equilibria and stability of proteins and therefore tracking protein conformations inside their native environment, the cell, is currently a challenge which is addressed by a number of biophysical methods such as NMR [1-4], FRET (Förster resonance energy transfer) [5]. Pulse dipolar electron spin resonance (PD-EPR) techniques, particularly by double-electron electron resonance (DEER, also called PELDOR) can also be used for in-cell structural studies. DEER measures the dipolar interaction between two paramagnetic centers, which is proportional to  $1/r^3$ , *r* being the distance between the two paramagnetic centers [6]. In the context of proteins, which are usually diamagnetic, DEER relies on introducing a pair of spin labels at well-defined positions in the protein, using site directed spin labeling, and it provides the distance distribution between the two labels. The most common method for spin labeling is via conjugation to the thiol group of native or engineered cysteine residues [7] or via conjugation to an unnatural amino-acid introduced at the DNA level at specific sites in the protein [8, 9]. Low temperature, in-vitro (frozen solutions) DEER measurements on proteins are currently fairly routine and the most commonly used spin label are based on nitroxide free radicals, with MTSL (S-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate) being the most popular reagent [6]. The beauty of DEER is its insensitivity of to the size of the molecule under study and the lack of strong environment background signals, which makes it an attractive method for in-cell structural studies. The transition from in-vitro to in-cell measurements is challenging because 1) both

the labeling scheme and the spin label have to be resistant to the reducing environment of the cell, 2) efficient methods for internalizing the labeled protein into the cell are essential and 3) the measurement sensitivity should be high to allow access to protein concentrations that are close to physiological concentrations (usually < 1  $\mu$ M).

The first pioneering in-cell distance measurements were carried out on a protein [10] and nucleic acids [11, 12], labeled with the common nitroxide spin labels based on the proxyl group  $(1-\lambda^1$ -oxidanyl-2,2,5,5-tetramethylpyrrolidine) and injected into Xenopus oocytes. All these works reported on the reduction of the nitroxide moiety of the proxyl group to diamagnetic hydroxyl amine in the cell (half life of about 50-60 min) [12]. This a serious obstacle for the development of in-cell DEER using the standard nitroxide-based spin labels and it gave us the idea that Gd(III) can be a good alternative for in-cell DEER measurements because of its redox stability and high EPR sensitivity at high frequencies [13-15]. In this short personal account I describe the developments that were essential for the realization of in-cell Gd(III)-Gd(III) DEER on proteins in my lab. These can be divided into 5 categories: (1) Instrumental developments, (2) feasibility studies with model compounds (rulers), (3) understanding the spin physics of a pair of Gd(III) labels and improving measurement methodologies, (4) protein labeling with Gd(III) tags and interpretation of the distance distribution obtained and (5)in-cell protein delivery, cell biology control experiments and finally, demonstration of in-cell DEER measurements. These different categories represent a wide range of expertise in different fields and therefore we relied on a number of invaluable collaborations. In the following I will give a brief description of each of these categories, which I envision as stations on the long and winding (but fun) road to incell DEER with Gd(III), although in reality they did not occur in a chronological order.

#### Instrumentation

About two decades ago we designed and built a W-band (95 GHz) pulse EPR spectrometer [16] inspired by the developments in the Moebius [17] and Schmidt [18] labs. Since then our W-band spectrometer has been upgraded several times [19, 20] and the most recent development has been the introduction of an arbitrary waveform generator (AWG), which allows the application of shaped pulses [21]. This latest development followed the work of Prisner [22, 23], Jeschke [24, 25] and coworkers, who introduced pulse shaping to EPR in general and DEER in particular. Our spectrometer is controlled by the software SpecMan [26] developed by Boris Epel and others and we enjoy the continuous support of Boris, adapting the software to our changing spectrometer.

The W-band frequency is well suited for EPR investigations of Gd(III). Gd(III) is a half integer, high spin (S = 7/2) ion with an isotropic g-factor, the spin density is highly localized on the Gd(III) ion and it's zero field splitting (ZFS) is relatively small, having an axial parameter, D < 40 mT [27]. Gd(III) complexes in frozen solutions exhibit a large distribution of the ZFS parameters. The spectrum of Gd(III) consists of 7 transitions and its central transition,  $|-1/2\rangle \rightarrow |1/2\rangle$ , is unique in the sense that it depends on the ZFS parameters only to second order and therefore it exhibits a low anisotropy. The width of this transition,  $\delta$ , narrows with increasing spectrometer frequency,  $v_0 (\delta \propto D^2 / v_0)$  [28], thereby increasing its EPR sensitivity. We realized the high sensitivity of Gd(III) at W-band following a close collaboration with Arnold Raitsimring, who was studying the coordination sphere of Gd(III) MRI (magnetic resonance imaging) contrast agents using W-band ENDOR (electron-nuclear double resonance) [29, 30]. This was in the mid 2000's, when DEER distance measurements using nitroxide spin labels began attracting considerable attention and many labs became engaged in the development of the technology, the theory required for data analysis and applications to bio-molecules. These developments led Arnold Raitsimring to suggest that W-band DEER on a pair of Gd(III) ions would benefit from high sensitivity. The realization of this idea led us to the next station.

#### Model compounds

Once the idea of Gd(III)-Gd(III) DEER was in the air, the next step was to demonstrate the

#### **Guest of the issue**



Figure 1. (a) The first bis-Gd(III) ruler, (b) W-band, 10 K, primary DEER trace of the ruler shown in (a) and (c) the derived distance distribution [13].

feasibility of such a measurement on a rigid model compound with a well-known Gd(III)-Gd(III) distance. At that time, the lab of David Milstein at the Weizmann Institute was collaborating with the Degani lab at Weizmann, which was engaged in MRI, and synthesized a derivative of estrogen conjugated to a Gd(III) contrast agent targeting the estrogen receptor for molecular magnetic resonance imaging [31]. I approached David Milstein and very enthusiastically asked him if he could extend these efforts and make for us a bis-Gd(III) ruler for testing the feasibility of Gd(III)-Gd(III) DEER measurement. Fortunately, he agreed and designed and synthesized the ruler shown in Fig. 1, with a Gd(III)-Gd(III) distance of 2.1 nm. At that time, based on DEER results on nitroxides, we thought that a short distance around 2-3 nm will be the easiest to measure ; in retrospect this was a naive thought. DEER measurements were carried out at W-band in my lab and at Q-band in the Raitsimring lab [13]. We assumed that because the spectrum is dominated by the central transition and the pump pulse induces only  $\Delta m_s = \pm 1$  transitions, we could treat the system as an effective S = 1/2 system and therefore used the DeerAanalysis software [32], developed for a pair of S = 1/2 spins, to analyze the data. This analysis gave a distance distribution with a maximum that was in agreement with DFT calculations [13] and with the crystal structure that came out later [33]. The width of the distance distribution was significantly larger than expected and the modulation depth was low, but at the time we were very happy with the

correct distance and did not pay too much attention to the width. This work also stressed several additional attractive features of Gd(III) in the context of distance measurements: (1) The high spin of the Gd(III) ion leads to a four-fold larger effective amplitude of the irradiating microwave field,  $B_1$ , for the central transition of Gd(III) than for nitroxides (S =1/2). This allows, accommodating short pump pulses also on spectrometers with limited microwave power. (2) The spin-lattice relaxation time of Gd(III) is short, allowing rapid signal averaging at low cryogenic temperatures. (3) The central transition can be considered as effectively isotropic because of the large ZFS distribution (in amplitude and orientation) and this abolishes the orientation selection, which complicates nitroxide DEER data analysis at high fields [34].

Later on, Adelheid Godt and coworkers designed and synthesized a series of water soluble bis-Gd(III) rulers, (Gd-PyMTAspacer-Gd-PyMTA), comprising the same Gd(III) chelate shown in Fig. 1, covering Gd(III)-Gd(III) distances of 2-8 nm [35]. W- and Q- band DEER measurements followed by data analysis using the standard data analysis approach employing DeerAnalysis gave distance distributions of which the absolute maxima agreed very well with the expected distances. However, in the case of the short distances of 2.1 and 2.9 nm the distance distributions revealed broadening and additional peaks, similar to the observation on the first ruler. This was also clearly manifested in the distortion of the Pake pattern obtained from the Fourier transform (FT) of the DEER trace after background removal. This led us to look more carefully into the simplified data analysis approach we were applying and to consider explicitly the effects of the high spin and the ZFS on the DEER frequencies.

## Gd(III) spin physics and measurement methodologies

Second order perturbation theory treatment of a spin Hamiltonian describing two S = 7/2spins with isotropic g-values and different ZFS tensors showed that the distortions observed for short distances are a consequence of the contributions from pseudo-secular terms of the dipolar Hamiltonian [35]. The contribution of these terms is negligible under the weak coupling approximation, namely when the frequency difference of the two spins is much larger than their dipolar coupling frequency. For nitroxides this frequency difference is determined by the frequency separation of the observe and pump pulse,  $\Delta v$ . In analogy, in Gd(III) the central transitions of the two dipolar interacting spins are separated due to differences in the D value (because of the large distribution of D values) or different orientations with respect to the magnetic field. When this separation is small, the weak coupling approximation no longer applies and the pseudo-secular terms lead to state mixing of the  $|-1/2, 1/2\rangle$  and  $|1/2, -1/2\rangle$  levels of the two spins and consequently to a shift in the frequency of the dipolar oscillations when any of these levels are involved in the DEER experiment. As a consequence, data analysis with the commonly used DeerAnalysis software [32], which is based on the weak coupling approximation, yielded distance distributions that are artificially broadened and include spurious peaks to fit the ensuing distorted Pake pattern. Numerical simulations were carried out in the frequency domain, taking into account the dipolar pseudo-secular terms and the ZFS, reproducing the experimental observations [35]. This theoretical treatment predicted that these undesirable effects increase with decreasing Gd(III)-Gd(III) distance and D value and will not be significant when setting the observe and/or the pump pulse to higher m<sub>s</sub> transitions. This was later confirmed by exact time-domain simulations of Gd(III)-Gd(III) DEER by explicit density matrix propagation to elucidate the factors shaping Gd(III) DEER traces (in collaboration with Ilya Kuprov) [36]. The theoretical understanding of the spin physics behavior led to the introduction of experimental measures that mitigate the difficulty encountered at short distances for small ZFS.

#### **Guest of the issue**

We demonstrated experimentally on a Gd(III) ruler that by increasing the frequecy difference between the pump and observe pulses  $\Delta v$ , from the standard 100 MHz to 1.09 GHz, the distortions were reduced, the Pake doublet was recovered, and the expected narrow distance distribution, with a maximum at 2.35 nm was obtained [37]. The increase in  $\Delta v$  results in an increase in the contribution of the individual  $|-5/2\rangle \rightarrow |-3/2\rangle$  and  $|-7/2\rangle \rightarrow |-5/2\rangle$  transitions to the observed signal at the expense of the  $|-3/2\rangle \rightarrow |-1/2\rangle$ . Although such a setting compromises sensitivity, it can be tolerated because a short evolution time is sufficient for short distances. Alternatively, one can choose a Gd(III) spin label with a larger ZFS.

Another way to reduce the effect of the dipolar pseudo-secular terms is to carry out RIDME (Relaxation-Induced Dipolar Modulation Enhancement) measurements [38, 39]. In this experiment the pump pulse is replaced by a fixed, relatively long time interval during which the magnetization is along the z axis and the spins undergo spontaneous flips due to spin lattice relaxation. As the spin flips are not restricted to the central transition but are experienced by spins throughout the Gd(III) spectrum the relative contribution of the pseudo-secular terms is reduced significantly and the derived width of distance distribution should be close to the real one. This has been demonstrated on a bis-Gd(III) ruler with a very small ZFS and a distance of 2.35 nm [40]. A drawback of this technique for high spin systems such as Gd(III) is the appearance of harmonics of the dipolar interaction frequency arising from double and triple quantum relaxation pathways [39]. These harmonics can be accounted for by adding two additional fit parameters to the DEER analysis procedure [41]. We found RIDME very useful as a complementary technique to Gd(III) DEER to detect artificial broadening [40]. Finally, we note that for systems exhibiting inherently broad distance distributions, the artificial broadening due to the deviations from the weak coupling approximation will be masked by the intrinsic broadening.

The phase memory time plays a crucial role in the DEER experiment because the maximal distance that can be accessed and the experiment sensitivity strongly depend on it. Therefore, understanding the mechanisms governing the phase relaxation of Gd(III) chelates in frozen glassy solutions is important. Together with Arnold Raitsimring [42] we observed that in addition to the usual mechanisms of phase relaxation known for nitroxide based spin labels, Gd(III) is subjected to an additional phase relaxation mechanism that features an increase in the relaxation rate from the central transition to the periphery of the EPR spectrum. Analysis of the data led to the formulation of a transient zero field splitting (tZFS) relaxation mechanism, which becomes dominant (or at least significant) when all relaxation mechanisms such as spectral diffusion caused by nuclear spin diffusion, instantaneous and electron spin spectral diffusion, are significantly suppressed by matrix deuteration and low concentration, and when the temperature is sufficiently low to disable spin lattice interaction as a source of phase relaxation. Additional insights on Gd(III) relaxation were reported by other labs [43]. As the ZFS is a key player that dictates the performance of Gd(III)-Gd(III) distance measurements, understanding how it is determined by the Gd(III) coordination environment is important and progress in this direction has been recently reported [44].

Currently, the the standard Gd(III)-Gd(III) DEER set up in the lab is to apply chirped pulses situated symmetrically on both sides of the central transition, while setting the observe pulse to the central transition [21, 24, 45]. This leads to a significant SNR increase, depending on the width of the central transition.

#### Protein labeling with Gd(III) tags and "in-vitro" DEER

Once the feasibility of Gd(III)-Gd(III) DEER has been demonstrated and its related funda-

mental spin physics has been understood the next step was to demonstrate that it is indeed useful in terms of the information it can give on biomolecules. Historically, demonstration of Gd(III)-Gd(III) DEER in proteins was reported as early as 2010 (see Fig. 2a) [14], before a complete understanding of DEER with half integer, high spin systems had been achieved. Actually, it was the realization that Gd(III)-Gd(III) DEER can be applied to proteins [14, 46] and nucleic acids [47] that served as a driving force for the synthesis of the series of rulers and the associated theoretical studies [35]. In this context one has to develop the appropriate labeling scheme and the associated Gd(III) chelating tags that can bind the protein efficiently and site-specifically. The large knowledge accumulated from paramagnetic NMR with lanthanide tags is of great benefit. In this front we enjoyed a wonderful, long term collaboration with Gottfried Otting, a world leader in paramagnetic NMR, which resulted in 17 co-authored publications [9, 14, 15, 37, 40, 46, 48-58] that established Gd(III) as a spin label for EPR-based distance measurements, along with the first demonstration that Mn(II) can be used as well, followed by other works [48, 55, 59, 60]. Most of these works employed a labeling scheme based on native or genetically engineered cysteines, where the conjugation to thiol groups generates a S-S bonds. This also includes a double arm Gd(III) tag [15], designed in the spirit of the



**Figure 2.** (a) W-band DEER results (25 K) for  $\tau_{C}$ 14 doubly labeled with Gd(III)-4MMDPA (shown on the right) at C553 and S528C. (left) Primary DEER time domain data before and after background removal and the corresponding superimposed calculated trace obtained with the distance distribution shown in the center panel [14]; (b) left: W-band DEER data of C54T/C97A/D72C/R76C/D127C/V131C T4 lysozyme labeled with the double arm Gd(III) spin label shown on the right, after background removal and the corresponding superimposed calculated trace (red) obtained with the distance distribution shown in the center panel [15].

double arm Rx nitroxide spin label [61], which attaches to two cysteine residues situated 4 amino acids apart and thus minimizing the label flexibility. An example is given in Fig. 2b, where long lasting modulations and a narrow distance distribution are observed. Additionally, it has been shown that distances of 6 nm can be readily accessed via Gd(III)-Gd(III) DEER because of the relatively long phase memory time [46]. Importantly, many of the proteins used to demonstrate the performance of Gd(III)-Gd(III) had crystal structures and it has been shown that experimental distance distributions agreed rather well with those predicted by a model developed by Thomas Huber, which uses the crystal structure and takes into account the physicochemical properties of Gd(III) [49].

A different labeling approach is to use a conjugation chemistry which generates a C-S bond with the protein. Tags for DEER developed by the lab of our collaborator Xun-Cheng Su followed this labeling approach [59, 62, 63]. Another cysteine independent labeling scheme employs conjugation to unnatural amino acids via click chemistry [12, 52, 56]. Currently there are more than a dozen Gd(III) tags that have been employed in DEER measurements in my lab and new ones are being designed by others [64, 66, 67]. Gd(III)-Gd(III) has been successfully demonstrated also on membrane proteins [36, 68]. Furthermore, Gd(III)-Gd(III) DEER has been shown to be rather insensitive to multi-spin effects because of its low modulation depth [68]. This extensive volume of Gd(III)-Gd(III) distance measurements in proteins in frozen solutions forms the essential basis for the next step - in cell distance measurements.

## In-cell protein delivery and in-cell DEER

The transition from solution to in-cell distance measurements is not a simple one because it introduces new requirements already mentioned in the introduction. Among the labeling schemes mentioned in the previous section, the formation of di-sulfide bonds is inappropriate for in-cell measurements because these will be reduced and cleaved in the cell. The other two schemes are suitable. So far we have used the C-S conjugation route with four different Gd(III) tags, shown in Fig. 3. Gd(III)-maleimide-DOTA (DOTA-M), which is commercially available, has a small ZFS and a narrow central transition, important for high sensitivity. It's drawback is it's flexible tether that may introduce broadening to the distance distribution. Another tag, which is compact and rigid, Gd(III)-PyMTA, preforms very well in solution but has an insufficiently high Gd(III) binding constant, which under certain conditions can lead to leakage of the Gd(III) from the labeled proteins [69]. The two other tags, BrPy-DO3MA and BrPy-DO3A, feature a multi-dentate cyclene group, like DOTA-M, which ensures a very high binding constant, the same rigid linker to cysteines but they differ in their ZFS and phase relaxation. BrPy-DO3A has a smaller ZFS and a longer phase relaxation, which leads to more intense EPR signals and higher modulation depth, and is therefore superior to BrPy-DO3MA [62, 63].

Our first in-cell Gd(III)-Gd(III) DEER on a protein was demonstrated on ubiquitin labeled with Gd(III)-DOTA-M, which was introduced into the cell via hypotonic swelling [70], a method we learned in the laboratory of Silvio Aime. The same tag was used in the study of the oligomeric state of  $\alpha$ -synuclein in the cell [2]. In this work the DEER measurement complemented extensive in-cell NMR measurements in collaboration with the laboratory of Phil Selenko and the labeled protein was delivered into the cell by electroporation. Two doubly labeled Gd(III)-DOTA-M mutants of a-synuclein were prepared and comparison of the in-cell and in solution Gd(III)-Gd(III) DEER showed that the protein was highly disordered in both environments. In addition, two singly labeled Gd(III)-DOTA-M a-synuclein mutants delivered into the cell gave no DEER effect, indicating that in the cytoplasm of HeLa cells a-synuclein is monomeric. This was an important experiment that ruled out the presence of significant amounts of oligomeric  $\alpha$ -synuclein, which may have escaped NMR detection due to their slow tumbling. Because  $\alpha$ -synuclein is a highly flexible protein the use of Gd(III)-DOTA-M did not compromise distance resolution, yet the experiment benefited from high sensitivity.

We used the same label to study the conformational equilibrium of calmodulin (CaM) in solution, in cell extract and in HeLa cells. CaM is a Ca<sup>2+</sup> ion sensing protein, which regulates the function of many other proteins [71]. It's structure comprises globular N- and C-domains connected by a flexible linker. Each domain contains a pair of Ca<sup>2+</sup> binding sites, where the uptake of Ca<sup>2+</sup> triggers conformational changes that enable binding to target proteins with high affinity and thereby modulating their activity [71]. The binding of the target protein is also associated with a large conformational change. To detect variation in the inter-domain distance we placed the label in the N- and C-terminal domains. We compared the distance distribution obtained in solution for CaM labeled with rigid Gd(III)-PyMTA and flexible Gd(III)-DOTA-M and found for both comparatively broad distance distributions. As the distances were rather long ( $\approx 5$  nm) we chose to work with Gd(III)-DOTA-M for sensitivity considerations. The solution distance distributions of apo-CaM and holo-CaM in the presence and absence of the IQ target peptide could be described by combinations of closed, open,



**Figure 3.** Chemical structures of Gd(III) spin labels suitable for in-cell Gd(III)-Gd(III) DEER. In all labels Gd stands for Gd(III). After ligation the two bottom labels are referred to as Gd(III)-BrPy-DO3MA and Gd(III)-BrPy-DO3A.



**Figure 4. (a)** In-cell W-band echo-detected EPR spectra of ubiquitin D39C/E64C labeled with BrPy-DO3A-Gd(III); **(b)** chirp-pulse DEER data after background correction and the fits (gray) obtained with the distance distributions derived shown in **c** measured on cells frozen 5 and 20 h after delivery. The blue traces in panels (**b**) and (**c**) correspond to ubiquitin D39C/E64C labeled with BrPy-DO3MA-Gd(III) (labeled as DO3MA in the figure) for cells frozen 5 h after delivery [62].

and collapsed conformations. In cell extract, *apo-* and *holo-*CaM binds to target proteins in a similar way as *apo-* and *holo-*CaM binds to the IQ model peptide solution. In HeLa cells, however, in the presence or absence of elevated in-cell Ca<sup>2+</sup> levels, CaM unexpectedly produced more open conformations and very broad distance distributions indicative of many different interactions with in-cell components, quite different from cell extract.

We demonstrated the superiority of Gd(III)-BrPy-DO3MA and Gd(III)-BrPy-DO3A over Gd(III)-DOTA-M in terms of distance resolution (width of the distance distribution) on ubiquitin (Fig. 4) [62]. Here we showed that in-cell Gd(III)-Gd(III) DEER on Gd(III)- BrPy-DO3A labeled ubiquitin and GB1 can be measured on cells frozen 20 h after delivery by electroporation, indicating the high stability of the linker to the protein and the tag, as well as the high sensitivity. Using DEER with chirped pulses an in-cell protein concentration of about 5  $\mu$ M, DEER traces with an evolution time of 4  $\mu$ s could be collected overnight.

#### Outlook

I have described here the stations we have passed, which I estimate to be half way through the long and winding, but fun road to routine applications of in-cell Gd(III)-Gd(III) DEER. We feel that the methodology development has reached a state which is sufficient for applications of in-cell DEER to what we refer to as a "real" biological question. I am happy to see other groups (Un, Lovett and Bordignon Labs) joining in with new labels and new strategies that will further advance this approach. In parallel, there are other important efforts in developing in-cell distance measurements by EPR using bio-resistant trityl and nitroxide spin labels, broadening the scope of in-cell EPR distance measurements. Eventually we will be able to use two non-identical spin labels (using orthogonal labeling methods), which will further increase sensitivity and will break the limit of one distance per sample and will permit three [55] or even more distances per sample. Another approach based on central transition linewidth in continous wave EPR spectra at very high frequency is promising and should be further developed [72].

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#### **Guest of the issue**

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<text>

Potable ESR instrument : ESR-X10SB

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## 30 Years of the International EPR Society

## **EPR** instrumentation

Gareth R. Eaton University of Denver

PR was developed as a means of mea-Esuring electron spin relaxation times. Theories of spin relaxation developed prior to the discovery of EPR described spins in a crystalline lattice (Waller, 1932; Van Vleck, 1940). Consquently, many of the early applications, following the Zavoiski demonstration of resonance in liquid solutions (1944), were of metals in crystals, either as pure compounds or diluted in a diamagnetic host. The early use of transmission resonators was an application of technology for gas-phase microwave spectroscopy, which the Bleaney lab had performed extensively. A measurement of ferromagnetism was recognized as demonstrating resonance, which immediately led to measurements of paramagnetic resonance. Since theories of the electronic energies and relaxation of 3d transition metals were most fully developed, and lanthanides were of intense interest, hundreds of these samples were measured in the first few years, especially by the Oxford lab of Bleaney (see the picture of Bleaney's magnet in another article in this newsletter). It was natural to add paramagnetic organic free radicals to the set of samples of interest, and fairly quickly investigators found hyperfine splittings (Weissman, 1953) and identified dependence of spectra on concentration, oxygen, and many other parameters.

Early labs built their own instruments, rapidly selecting X-band reflection resonators, magnetic field modulation, lock-in detection, hybrid T and then circulators and reference arm to improve signal-to-noise.

The first commercial EPR spectrometer was the Varian V4500, marketed in 1956. Several other commercial vendors followed, and for a while there were multiple commercial EPR spectrometers.

In 1965 Varian produced the E3, which was a small, transistor-based spectrometer. The Bruker spectrometer produced in 1967 was also transistor-based. Current students may not recognize the major step in reliability (and size) that accompanied the change from vacuum tubes to transistors. The extensive use Figure 1: V-4502 EPR Spectrometer System from the early 1960s, showing spectrometer console, 100 kHz field modulation and control



unit (on top of console), and six-inch magnet with multi-purpose cavity installed. Also shown are the microwave bridge and graphic recorder. (picture reproduced from Varian sales literature.)

of computers today (which started in the early 1970s) obscures the great importance to magnetic resonance (NMR as well as EPR) of the strip-chart, and then the flat-bed, recorder.

Signal-to-noise improvements over the ca. 63 years since the first commercial EPR spectrometers (ca. 1956) have extended the applications of EPR to qualitatively different regimes. One measure of the improvement in sensitivity is that the famous Cummerow and Halliday (1946) paper measured the EPR signal from 173 g of  $MnSO_4 \cdot 4H_2O$ . The earliest specification of signal-to-noise (S/N) performance of an EPR spectrometer was 20 for a weak pitch sample in the Varian V4500 in 1960. The weak pitch S/N measurement became the standard for intercomparison of X-band CW spectrometers. Using TE<sub>102</sub> cavity resonators, improvements in electronics resulted in an almost linear improvement over time to S/N = 300 on the Bruker ESP300 in 1989. Renewed interest in loop-gap resonators (Zavoiski used one!) in the 1980s stimulated creativity in resonator design. Subsequent improvements in resonators relative to the TE<sub>102</sub> (standard) cavity made possible dramatic improvements to S/N = 3000 in 2007 on a Bruker Elexsys with an SHQ cavity.

A major development in EPR at many frequencies is the ability to design resonators to fit the sample and match the experimental goals, such as pulse, rapid scan, distance measurements, relaxation time measurements, imaging, or very small samples. Spectroscopy and imaging of very small samples in materials



**Figure 2:** The EMXnano is a benchtop CW spectrometer that provides 6000 G field sweeps with sufficient sensitivity for many research applications. The spectrometer is fully calibrated for quantitative EPR. Photograph from Bruker sales literature.

science and biology provide new challenges to design the entire spectrometer for a particular application. Until recently, all EPR spectrometers looked the same except for styling – they consisted of a magnet, bridge, waveguide resonator, and console.

The need for benchtop, or smaller, EPR spectrometers has brought many vendors into the field, competing on specifications relevant to particular application fields. The Bruker EMXnano, for example, looks totally different from the large, versatile research spectrometers such as the Elexsys.

EPR requires much more stable sources, with lower phase noise, than most other microwave applications. This is why the Varian klystron was such an important part of early commercial spectrometers. Current X-band spectrometers use very carefully engineered Gunn diodes. A lot of work is needed at other

#### Anniversaries

frequencies to achieve sources that are as good as the X-band stabilized Gunn sources. Detection has used 100 kHz lock-in amplification since about 1960. Traditional EPR has used macroscopic magnetization changes at resonance, detected with a crystal or mixer for CW, pulse, and rapid scan. Methods for smaller numbers of spins use spin-sensitive AFM, optical, or electrical detection.

Cryogenic operation is important because so many species have very short relaxation times at room temperature. Early studies used liquid  $H_2$  and liquid He in quartz Dewars, which required skill to use. Varian advertised one in 1959 for \$2575, which was a large cost back then. The Oxford metal cryostats used with the Bruker FlexLine resonators were a major simplification for EPR. The high cost of liquid He makes the new "Stinger" closed cycle He cooling systems very attractive. One can now operate for weeks at a time at 5 K without using liquid He and using very little He gas.

Important applications are pushing EPR to both higher and lower field/frequency regimes. Some spin systems can only be observed at high field/high frequency. Studies relevant to NMR, DNP, and MRI require EPR at the same field strength as these NMR applications. In vivo studies require fields and frequencies lower than have commonly been used in EPR. Oximetry and the many biomedical measurements for which nitroxide radicals are being applied in vivo require EPR frequencies of 1 GHz and lower. In vivo imaging has great promise. Imaging of mice at frequencies between about 250 MHz and 1 GHz is being developed in several labs. Measurements of radiation dose to teeth has been achieved with a purpose-built dosimeter. Oxygen-sensitive India ink injected into humans can be used to monitor peripheral vascular disease.

EPR is poised to take advantage of modern advances in analog and digital electronics.

New digital technologies, especially arbitrary waveform generators (AWG) are revolutionizing pulsed and rapid scan EPR. Only the small market prevents investment of the resources needed to do the extensive engineering required to take advantage of many technologies demonstrated in academic laboratories, such as

- Rapid scan direct detection
- Digital signal detection
- Arbitrary waveform generator (AWG) source
- Pulse shaping
- Multiple harmonics for CW
- Micro EPR
- EPR on a chip
- Hyphenated techniques
- Superconducting magnets without liquid He

Research publications point to exciting information available from electron spins via:

- Very high field and high frequency spectrometers
- Small resonators for small samples (but large spectrometers)
- Large resonators for in vivo
- Small spectrometers for
  - Environmental applications
  - In-plant applications; process analysis
  - Teaching

Beyond X-band, Q-band (ca. 34 GHz) has proven to be the most favorable frequency for DEER measurements. W-band (94 GHz) spectrometers were popular for a while, but the cost of operation of the supercon magnets has dampened enthusiasm. Some steps toward commercial development have been made at higher and lower frequencies, such as 263 GHz pulsed EPR, and L-band (1 GHz) in vivo imaging. The interests in frequencies both higher and lower than X-band create opportunities for substantial engineering effort to improve S/N. Especially at high frequencies the published sensitivity performance is not as high as predicted by extrapolations from X-band.

Those who visited our lab this year saw the Bruker prototype X-band rapid scan EPR system, and also our implementation at low frequency, which is being done in collaboration with Bruker. Rapid scan EPR is a new development in EPR, incorporating some of the best features of CW and pulsed EPR. If a CW spectrum is obtained with modulation amplitude that is small enough to provide a first-derivative curve with high fidelity, the modulation amplitude should be less than about 10% of the line width. Thus only a small fraction of the amplitude of the spectrum is measured in each modulation cycle. In rapid scan measurements, in the common cases of spectra that are narrower than the field-scan amplitude, the entire spectrum amplitude is measured, not just 1/0 of it. In addition, if the relaxation time of the spin system is long enough that the CW spectrum can be power-saturated, higher B<sub>1</sub> can be used in rapid scan than in CW spectroscopy. For common organic radicals the resulting spectral amplitude can be larger by up to about a factor of 3 in rapid scan relative to CW just due to the power saturation differences. Up-field and down-field scans and I and Q signals can be combined. One can record several rapid field scan cycles in modern digitizers, providing the further advantage of a comb filter.



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## The Past, the Present and the Future of EPR Spectroscopy for Physics and Materials Sciences

#### **Christoph Boehme**

t the 2019 Rocky Mountain Confer-A ence for Magnetic Resonance in Denver in July 2019, we celebrated the 30th anniversary of the IES. On this occasion, I was asked to speak about some of the EPR related breakthroughs in physics and materials science since the IES's inception. I accepted, of course, being aware of the many achievements and discoveries that had come from this distinguished scientific community throughout all these years. Once I began to compile my presentation though, I realized soon that this would be a quite challenging task. I had to somehow squeeze 30 years of research into a 15 minute talk. To achieve this in a balanced way, I reached out to a few EPR researchers within the condensed matter physicists, materials science, engineering, and chemistry domains. I asked them about their views on what the most crucial developments had been. I received a lot of input – thank you to all who responded! Remarkably, among these statements, there was quite a significant overlap regarding the assessments of whom the real movers and shakers in materials science and physics-related EPR studies had been and which materials related EPR work ought to be remembered. The following is a brief summary of the picture that has emerged in the course of these exchanges.

In retrospect, it appears that in 1989, EPR spectroscopy was deemed to be a well-established, "complete" technique. The fundamental physical processes associated with EPR, including the physics of magnetic resonance, spin relaxation, and the quantum mechanics of electron and nuclear spin propagation, were considered to be well understood and readily available. Similarly, the technical aspects of EPR, i.e., the know-how to build EPR spectrometers and their components in the most efficient way, appeared to be well-established. Most EPR related materials research at the time focused on the study of point defects, predominantly semiconductors, but also on some dielectric materials [1]. Clearly, in 1989, the value of EPR for defect spectroscopy had been fully understood, given the breakthrough EPR studies of impurities in the 1950s and 1960s [2], electronically active defects at crystalline silicon/silicon dioxide interfaces [3] in the 1970s and amorphous semiconductors, most significantly hydrogenated amorphous silicon in the 1980s [4]. All this EPR related work had made significant contributions to the advancement of microelectronics and, thus, modern information and energy technologies. Since 1989, microelectronics has continued to evolve. In fact, it has accelerated following Moore's law and traditional EPR based defect spectroscopy has, unsurprisingly, remained an active field [5-7]. Since 1989, point defects and localized paramagnetic electronic states in organic lighting and display application [8-11] and high-power applications [12, 13] have been intensively studied with EPR related methods.

Beyond the continued pursuit of EPRrelated research areas that had already been established in 1989, several paradigm changes have taken place, which predominantly relate to the way EPR is used for physics and materials research. These developments were initially triggered by progress on EPR spectroscopy and its related methods, which, in turn, have significantly broadened the application range of EPR far beyond traditional spin spectroscopy.

One of the crucial moments for EPR related physics and materials breakthroughs past 1989 was the very first single-spin EPR detection. To my knowledge, this happened right in 1989 when the IES was born. Y. Manassen et al. [14] at the IBM Watson Research Center performed a scanning tunneling microscopy-based electrically detected magnetic resonance (EDMR) experiment on a single paramagnetic center in a crystalline silicon (111)/ silicon dioxide tunneling junction through an RF frequency sweep in the presence of a static magnetic field. The impact of this early single-spin EPR experiment did not really unfold until four years later when Joerg Wrachtrup [15] and coworkers combined single molecule detection with magnetic resonance in order to perform the first single-spin optically detected magnetic resonance (ODMR) experiment on a pentacene molecule. Even though ODMR had already existed for more than 30 years, following Geschwind's first demonstration in 1959 [16], this first ODMR-based single-spin detection experiment, led to a renaissance of EPR spectroscopy's status as a crucial experimental technique for physics research and spectroscopy development. Following this work, a variety of optical centers were scrutinized for single spin ODMR experiments and, eventually, nitrogen-vacancy (NV) centers emerged as prime candidates for the straight forward room temperature single spin-EPR detection [17]. As illustrated in the Figure adapted from Ref. [18], NV centers possess triplet ground states and an optical cycling transition, a combination that makes them almost unbeatable by other paramagnetic species for single-spin quantum information and sensing applications. Thus, by 2004, the detection of coherent single spin motion effects such as Rabi oscillation or Hahn-echo experiments on single NV centers (see the Figure), had became straight forward [18].

The availability of single-spin access led then to a paradigm shift for EPR spectroscopy. Rather than performing EPR spectroscopy on an unknown paramagnetic physical system in order to learn something about this system, EPR spectroscopy was now performed on an extremely well-known paramagnetic system, in particular a single paramagnetic state, in order to probe local magnetic fields and many other physical variables that can affect magnetic resonance (for instance by controlling spin relaxation times). Thus, EPR would no longer have to be a spectroscopy technique per se, but instead, it also could be a sensor technique for all kinds of physical variables [19, 20].

With these developments, a frenzy of studies of spin-selection rules and other processes in condensed matter started, leading to the demonstration of various indirect EPR detection schemes similar to ODMR. These activities included the development of new electrically detected magnetic resonance (ED-MR) schemes [21-23] (following the initial EDMR work in the 1960s and 1970s), magnetic resonance force microscopy (MRFM) [24], noise detected magnetic resonance [25] and even bird detected magnetic resonance [26] where the ability of European Robins to see geomagnetic fields was shown to be compromised under EPR of the free radical pairs responsible for this sense. Among these developments, EDMR and MRFM were also demonstrated to be suitable for single-spin



Figure: Reprinted figures of optically detected pulsed EPR of a single nitrogen vacancy center in diamond with permission from F. Jelezko, T. Gaebel, I. Popa, A. Gruber, and J. Wrachtrup.: Phys. Rev. Lett. 92:076401, 2004 [18] (https://doi.org/10.1103 / Phys. Rev. Lett. 92:076401). Copyright 2004 by the American Physical Society. (A) Energy level scheme of the nitrogen vacancy defect center in diamond with a paramagnetic ground state that has opened up countless avenues for single spin based sensing and quantum applications. (B) Optically detected Rabi oscillations of single electron spins in a single NV defect center are shown for two values of the MW Rabi frequency (solid grey lines represent the measured data, and the thick lines show the simulations) showing the Inherently long coherence times of NV centers.

EPR detection, yet, as for ODMR, only for paramagnetic electronic states in suitable physical host matrices and under suitable environmental conditions [27–29].

By the end of the 1990s, the developments of single spin readout schemes had also begun to affect the exploration of quantum information technologies that had emerged since the mid-1990 and that has continued ever since. The ability to read single electron and nuclear spin qubits suggested that spin qubits could form the backbones for these new information technology paradigms. Notable in this regard is Kane's 1998 electron/nuclear spin quantum computer proposal using phosphorous donor spin qubits in crystalline silicon [30] that was based on the realization made by Feher about 40 years earlier that these systems have tremendously long coherence times [2]. A variety of electron spin-qubits based on charge quantum dots also emerged [31, 32], many of which led to demonstrations for how

to control, read, and interact with their respective electron spin qubit systems [33–36]. After more than 20 years of work in this area [37, 38], it appears that the currently leading quantum computer architectures, albeit based on superconducting flux qubits rather than spins, have greatly benefited from all of the EPR related work, in particular when it comes to pulse sequences for qubit manipulation and quantum gate operation.

Finally, I want to point out that the fundamental overall progress on EPR methods, in particular in electronics, has also affected the ways by which researchers perform EPR spectroscopy today. With the advent of solidstate microwave amplifiers, arbitrary waveform generators and much faster computers with much larger memories, there has also been much progress on EPR spectroscopy technology, including EPR on a chip [39] spectroscopy, high magnetic field EPR spectroscopy [40], new microresonator development, and adiabatic pulse shaping [41]. These developments, of course, are important also for EPR spectroscopy in chemistry, biology, and other domains. However, they are driven largely by advancements of materials research for EPR spectroscopy as well as by progress of the development of spin- and EPR- based quantum sensing and quantum information processing. In essence, EPR methods development depends on progress in materials science as much as materials science depends on progress on EPR spectroscopy.

So what comes next? To me, looking back at the past 30 years suggests two things about the future: (i) We need to refrain from making guesses about upcoming paradigm changes. Significant game changes have always been unpredictable. (ii) Physics and Materials Science related EPR spectroscopy breakthroughs have always broadened the applicability of this technique, yet rarely made previous research areas obsolete. When EPR based sensing using

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point defects had its advent, EPR spectroscopy on point defects did not stop. It will be exciting to see what paradigm shifts EPR will bring for physics and materials research in the next 30 years. I would expect the evolution of spin-qubits and spin-based quantum information technologies will fully come to fruition in the next decade, a development which could have the potential to change our world like few other technological developments before.

I want to conclude this quick review of the past thirty years of EPR related physics and materials research by generalizing a quote made by single-spin detection pioneer Joerg Wrachtrup about NV centers - EPR spectroscopy used to be applied for materials research, but now, materials research is applied for EPR spectroscopy. That, I believe, wraps it up - let's see what happens next!

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# 40+ Years of the Symposium and the Evolution of Modern EPR

#### Sandra S. Eaton and Gareth R. Eaton University of Denver

The first 30 years of the EPR Symposium were described in Volume 18, no.2-3, of the Newsletter in 2008. 2019 was the 42nd Symposium, the 30th year of the International EPR Society, and the 75th anniversary of the discovery of EPR. Contributions to the celebration were made by Christoph Boehme, Harold Swartz, and Gareth R. Eaton in brief talks at the banquet, attended by over a hundred people. Christoph sketched the history of EPR in materials science by citing a multitude of important papers published in the past 30 years. Hal described contributions of his lab and other close colleagues to preclinical and clinical applications of EPR. Gareth displayed the importance of instrumentation to the development of EPR, not restricted to the past 30 years. See separate articles in this Newsletter by Christoph and Gareth.

The Denver meeting originally was part of the Rocky Mountain Conference on Analytical Chemistry, which was affiliated with the Society for Applied Spectroscopy. EPR and NMR were added in 1978. Over the years, NMR and EPR became the strongest parts of the conference, and areas of analytical chemistry faded away. In 2004 Kurt Zilm became President and Sandra Eaton became Treasurer of the Rocky Mountain Conference organization, which was spun off from the Society for Applied Spectroscopy. Paperwork to define the tax exempt status of the organization was completed in 2008. Eventually, the conference was renamed the Rocky Mountain Conference on Magnetic Resonance, and the NMR portion emphasized solid state NMR. The organizers of the NMR part of the meeting were eager to move the meeting from Denver to the mountains, which was done for the first time in 2006. They also decided that the NMR sessions would be on alternate years. For the early years of the EPR Symposium Gareth and Sandra Eaton organized the sessions. Beginning in 2007 there was a transition to an organizing committee. The EPR-only meeting was in Denver in 2013, and in Snowbird Utah in 2015. In 2017 it turned out that the ISMAR meeting was scheduled in Quebec the same week as the EPR Symposium was scheduled to be in Denver. This unusual event resulted in merging with the ISMAR meeting in Quebec in 2017. ISMAR led the organization and forced parallel sessions, which was unfortunate from our perspective, since we strongly believe in the value of cross-fertilization between areas of EPR. The EPR Symposium was back in Denver this year. It will be in the mountains (Copper Mountain), joint with NMR, in 2020, and back in Denver in 2021.

Very strong enrichment of the Symposium over the past decade has resulted from the enthusiastic organization by Christoph Boehme of contributions from materials science. Distance measurements by DEER, SIFTER and other pulse measurements are a major part of the Symposium, as effort continues to develop methods to narrow the distributions of distances and define the uncertainties in distances. Fortunately, there is increasing awareness that EPR provides insights not available from X-ray and cryoEM structures, and there is excitement about potential for obtaining biologically-important distances in cells. After many years in which there was little focus on relaxation times other than as a measurement method in oximetry, suddenly longer phase memory times are the central goal of research in quantum information processing.

Beyond the science per se, there are important social changes. After trying special sessions for young investigators, the Symposium now includes students and other young investigators in the regular lecture sequence. A much larger number of female scientists participate than in the early years of the Symposium. The poster sessions also include undergraduate students. Much emphasis is placed on poster presentations. Posters are on display for the full length of the Symposium, with two scheduled sessions for formal presentation that always continue past the scheduled time because of the intense discussions around the posters. Awards for best posters honor the student presenters. There has been a major turnover of contributors, as many whose names almost defined the field of EPR are replaced by a new generation of researchers. EPR clearly has a healthy and vigorous future.

The Symposium would not succeed without the support of several sponsors. This year, support was provided by Bruker BioSpin, National High Magnetic Field Laboratory, Avanti Polar Lipds, Bridge 12 Technologies, Clin-EPR, Cryogenic US, Element Six, Magnettech/Rotunda Scientific Technologies, and Virginia Diodes. This set of sponsors itself tells a lot about the evolution of topics discussed at the EPR Symposium.



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An important start to the EPR Symposium each year is the Bruker BioSpin EPR User Meeting. Bruker updates the community on new instrumentation developments, and shows applications that stimulate ideas of experiments made possible by the new instruments. When the meeting is in Denver, there is an Open House with demonstrations in the Eaton lab. This year, for example, Bruker announced the X-band rapid scan accessory, and demonstrated it in operation on an E580 in the Eaton lab.

Shared EPR (led by Prof. Gary Gerfen) conducted workshops in conjunction with the EPR Symposium in 2015 (EPR on a Chip: Development and Applications of Micro EPR) and 2016 (pulse shaping).

Preceding the EPR Symposium this year, Shared EPR, the International EPR Society, and Bruker BioSpin sponsored an introductory EPR Summer School at the University of Denver. From over 200 applicants, 40 were selected. Lectures by five leading EPR researchers were followed by nine parallel small-group discussion or experimental tutorials. Bruker brought two EMXnano spectrometers so that we could have 5 parallel hands-on sessions for groups of 3 or 4 students in the Eaton lab.

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#### **Conference reports**



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Impressions from the EUROISMAR Conference in Berlin, 25–30. August 2019

The Free University in Berlin hosted the joint meeting of the 21st ISMAR and 15th EURO-MAR conference along with the German Magnetic Resonance Discussion group meeting in Berlin from August 25 to 30, 2019. 1,100 participants from all over the world gathered in the Henry Ford building and the Harnack house of the Free University for a colorful program covering the recent advances in magnetic resonance talks on MOUSE and low-field NMR, intrinsically disordered proteins, in-vivo methods, solution NMR methods, EPR, membranes, materials, physics, modeling of biological processes, carbohydrate interactions, biomolecules, dynamics, computation, hyperpolarization, NMR in Drug design, small molecules, and instrumentation. Peter Wright and Jane Dyson were awarded the ISMAR Prize for their work on intrinsically disordered proteins, Daniella Goldfarb and Angela Gronenborn received the Ernst Prize

in plenary lectures and fife parallel sessions. The local organizer Hartmut Oschkinat and his team created a unique and comfortable atmosphere embedding the scientific program arranged by Jeffrey Reimer in musical performances by young professionals and some attendees of all ages. Plenary prize sessions framed the invited and contributed of the EUROMAR Ampere division, Franz Hagn the Bloch lecture award of the German Magnetic Resonance Discussion Group, and Malcolm Levitt gave the Callaghan Lecture. The fact that three societies met together attracted so many participants, that registration had to be capped well before the meeting. A noteworthy event was the formal launching of an affordable non-profit, gold open access journal of the Ampere Society, entitled "Magnetic Resonance", for which editors in chief Geoffrey Bodenhausen and Gottfried Otting are now accepting the first manuscripts to undergo public reviewing. Although the distance between the two neighboring locations of the event, albeit close, at times gave rise to second-order perturbations, the overwhelming impression the attendees carried home was one of a broad range of lively and innovative science in our international magnetic resonance community embedded in the unique flair of Berlin today. The latter found its highlight in the conference dinner event at Clärchens Ballhaus, a historic, 100-year old dancing restaurant.

Bernhard Blümich

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confirmation of the manifestation of spin exchange in EPR spectroscopy. The author unfolds the details of comprehensive state of the art of theoretical calculations, which have been proven to become the core of the paradigm shift in spin exchange and set the direction for the future of spin exchange research. The book refers to important experimental data that confirms the theory. It describes the modern protocol for determining the bimolecular spin exchange rate from the EPR spectra, which will be especially interesting for experimentalists. Given its scope, the book will benefit all researchers engaged in theory and experiments in the area of spin exchange and its manifestations in EPR spectroscopy, where many remarkable applications of the spin probe have been developed.

#### POSITIONS

#### Silicon Quantum Technology EPR Technician Position

The Silicon Quantum Technology group (http://www.sfu.ca/physics/siliconquantum), based at Simon Fraser University's campus in Burnaby, Canada, has an immediate opening for an EPR Technician. This is a full-time, grant-funded position for an initial contract period of one year, renewable for an additional three years, at a starting salary rate of \$69K CAD p.a. plus benefits.

The primary responsibilities of the successful candidate will be to design, purchase components and test equipment for, assemble, build, code, and maintain a combination EPR/ ENDOR spectrometer to be used by the Silicon Quantum Technology group to perform their research. This spectrometer will be used to apply advanced CW and pulse sequences to samples often held at cryogenic temperatures and in concert with external equipment such as pulsed and/or CW optical and electrical signals. The data resulting from these experiments may be in the form of traditional EPR measurements or other signals such as electrical or optical measurements. Home-built or customized open-source software will be constructed to coordinate the delivery of these sequences and capture the resulting data, and this software will have an intuitive user interface, with live and saved data display options, and it will organize data for later retrieval.

The ideal candidate will have a Bachelor's degree in Physics or Engineering with courses or formal training in microwave (MW), radio-frequency (RF) and DC electronics/ electrical technology, computing systems and programming, mechanical systems, and the use of machine tools and related equipment and a minimum of 4 years related work experience.

Full details are available at https://www.sfu.ca/ physics/research/groups/ silicon-quantum-technology/research-EPR-technician-job.html.

All qualified candidates are encouraged to apply; however, Canadian citizens and permanent residents will be given priority.

Contact: Dr. Stephanie Simmons, s.simmons@sfu.ca, 778-782-3673

#### Bruker EPR Opening

The EPR Division of Bruker BioSpin has two openings for the Billerica, MA, USA office. Application Scientist

– Ph.D. in the following areas is a prerequisite for this position: Chemistry, Biochemistry, Physics, Molecular Biology.

Postdoctoral experience is desirable.
U.S. Citizenship or U.S. Permanent Resident status required.
The official posting is at: https://englishcareers-bruker.icims.
com/jobs/7800/epr-application-scientist-bruker-biospin-epr-team/job?hub=
12&mobile=false&width=940&height=
500&bga=true&needsRedirect=
false&jan1offset=-300&jun1offset=-240

Field Service Engineer

Minimum 3 years Life Sciences Field Service work experience diagnosing and repairing complex mechanical, electromechanical and/or electronic equipment required with demonstrated mathematical abilities.

Bachelor Degree desirable in Electrical Engineering, Electronics, Chemistry related fields.
U.S. Citizenship or U.S. Permanent Resident status required.

The official posting is at:

https://englishcareers-bruker.icims.com/ jobs/7795/field-service-engineer---brukerbiospin-epr/job?hub=12

## PhD position: DNP/MAS NMR using photo-excited triplet states

The research group of Dr. Guinevere Mathies in the Department of Chemistry at the University of Konstanz is looking for an enthusiastic PhD student to join our international team. He or she will investigate if and how the high polarization generated in the photoexcited triplet states of organic molecules can be used for Dynamic Nuclear Polarization

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(DNP). This project is part of a larger effort in our group to develop new forms of DNP to enhance the sensitivity of high-resolution magic-angle spinning (MAS) NMR.

The candidate we look for has a MSc degree in Physics, Chemistry or a related field and research experience in EPR spectroscopy is very welcome. He or she is highly motivated, driven by curiosity, and can work independently as well as in a team.

The PhD position is planned for 3 years with the possibility of extension and a salary according to the German TVL E13 67% scale. It is available immediately and applications will be considered until the position is filled. To apply, send a cover letter and a CV to Dr. Guinevere Mathies at guinevere.mathies@uni-konstanz.de.

For more information, visit our website: https://www.chemie.uni-konstanz.de/mathies

#### EQUIPMENT

Available: Used Varian EPR equipment (1) Varian E-104 EPR spectrometer with vertical style bridge and e-line fieldial. (2) Varian E-9 EPR spectrometer. Both available with warranty and continued service support. (3) Varian TM cavity with flat cell holders and flat cells. (4) Varian E-257 variable temperature controller with heater sensor and insert holder. (5) Varian E-272B field/frequency lock accessory.

Please contact: James Anderson, Research Specialties, 1030 S. Main St., Cedar Grove, WI 53013, USA. phone: 1-920-889-3893, e-mail: janderson36@wi.rr.com

EPR parts, electronics and hardware

Pulse generators, amplifiers, frequency counters, etc. We also offer X-band cavities, waveguide, klystrons, cells, etc. for Varian instruments. **Please contact:** techepr03@gmail.com for

availability and pricing.

#### Walker Scientific Electromagnet

I have a Walker Scientific HF-12H Electromagnet available for donation. The magnet has 12" diameter flat pole faces and a 4.5 inch gap. It has a standard H-frame style yoke that rests on a cradle that positions the yoke at a 45 degree angle typical for EPR. The magnet is 30 years old and was always used with a closed, deionized water cooling system.

Contact: John McCracken mccracke@msu.edu, 517 151-1159





#### Fast and stable high temperatures - High Temperature VTU for EMXnano

From polymer degradation to reaction monitoring, often you need access to higher temperatures. The High Temperature VTU accessory for the EMXnano is designed for such situations. The compact design provides rapid temperature changes with high stability.

- Maximum temperature: 200 °C / 473 K
- Flow: 15 L / min at maximum temperature
- Dewar compatible with liquid nitrogen VTU

## Innovation with Integrity



## Magnettech MS 5000 ...now a member of Bruker!

*"I am delighted that Bruker has acquired our EPR business to provide the MS 5000 benchtop system and related dedicated EPR analysis solutions, as well as global service and support. Freiberg is pleased to continue an EPR supply partnership with Bruker."* 

Dr. Kay Dornich, Managing Director of Freiberg Instruments GmbH

## Magnettech MS 5000

Extending Bruker's benchtop EPR Portfolio

The new Bruker Magnettech MS 5000 is an innovative instrument combining high quality performance with an ease of use for researchers. It offers dedicated and tailored turn-key solutions for specific quality control applications as well as for medical and pharmaceutical R&D applications.

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## Innovation with Integrity