ESR Microscopy
Current Capabilities and Future Directions

Aharon Blank
Schulich Faculty of Chemistry, Technion – Israel Institute of Technology
Haifa, Israel
Outline

- ESR Microscopy (ESRM) – current systems architecture and specifications
  - Pulsed ESRM, RT, LT
  - CW ESRM, RT
- ESR Microscopy – examples from recent sensitivity and resolution experimental tests
  - Pulsed ESRM – room temperature
    - 35 GHz, solids and liquids
  - Pulsed ESRM – low temperatures, 17 GHz
- Biological applications
  - Oxygen concentration near cells
  - Superoxide in roots
  - Intracellular imaging
- Future directions
  - Better sample preparation with new spin probes
  - Sensitivity and resolution improvements
  - Surface superconducting resonators
  - “Exotic” structural biology applications
- Summary
- Acknowledgments
ESRM – current systems architecture and specifications: Pulsed ESRM

- (a) Control PC
- (b) Timing system cards
- (c) Digitizer/Averager
- (d) Analog output cards
- (e) GPIB card
- (f) Microwave reference source
- (g) Pulsed microwave bridge
- (h) 6-18 GHz Transceiver
- (i) Q band block convertor
- (j) Gradient drivers module
- (k) High voltage tracking power supplies
- (l) +625V (X&Y gradients)
- (m) -515V (X&Y gradients)
- (n) +625V (Z gradient)
- (o) High voltage pre-regulators
- (p) For X&Y gradients
- (q) For Z gradient
- (r) Gradient coils drivers
- (s) Small capacitor (X&Y gradients)
- (t) Big capacitor (X&Y gradients)
- (u) Z gradient 1
- (v) Z gradient 2 + FFL lock
- (w) Imaging probe
- (x) Magnetic field controller
- (y) Magnet power supply
- (z) Monitor Scope

Microwave bridge(s)
Gradient coils drivers
Additional gradient driver units
Supported imaging pulse sequences

- $90^0$ pulse
- $180^0$ pulse
- Echo

$\tau = 150 - 10,000 \text{ ns}$

$G_x$ pulse sequence:
$\tau_{px} = 150 - 1400 \text{ ns}$

$G_y$ pulse sequence:
$\tau_{py} = 150 - 1400 \text{ ns}$

$G_z$ pulse sequence:
$\tau_{pz} = 150 - 1400 \text{ ns}$
Pulsed ESRM – Main components

MW Bridge

1. Sig. gen. source
2. Driver & power divider
3. Quad phase shifter
4. Tx var. att.
5. Tx pre amp.
6. Tx sw
7. Tx power amp.
8. Tune mode combiner
9. Rx defense sw 1
10. Circulator
11a. Resonator
11b. Q-band up / down converter
12. Rx defense sw 2
13. LNA
14. Rx var. att.
15. IQ demod.
16a. Man phase shifter
16b. Base-band amps.
Signal out

10 GHz signal from / to 6-18 GHz bridge

 Tx branch

 Rx branch

1. Input circulator
2. Q-band up / down converter
3. Tx sw
5. Tx power amplifier
6. Rx protect sw 1
7. Circulator
8. Q-band resonator
5. Tx power amplifier
6. Rx protect sw 1
7. Circulator
8. Q-band resonator
Pulsed ESRM – Main components

Gradient drivers
Waveguide to coax adapter
Semi-rigid coax
Gradient coils
XY stages
Resonator and sample holder
1 cm
Quartz sample holder
Coupling loop
Resonator
Semi-rigid coax

Imaging probe – RT 35 GHz, solids
Resonator mode – RT 35 GHz, solids
Gradient coils – RT 35 GHz, solids

- X gradient coils
- Y gradient coils
- Z gradient coils
- DC coils
- Shield

12 mm

y & z gradient coils (Golay)  y & z gradient coils (Maxwell)

Gold shield

12 mm
Rexolite sample holder, RT 35 GHz, liquids
Imaging probe – LT 17 GHz, solids
Imaging probe – LT 17 GHz, solids
Control software – Pulsed ESRM
<table>
<thead>
<tr>
<th>Tau</th>
<th>Def. start</th>
<th>Echo start</th>
<th>Averages</th>
<th>Repurate</th>
<th>Duty cycle</th>
<th>Round</th>
<th>Robbins</th>
<th>Total Averages</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>510</td>
<td>530</td>
<td>4</td>
<td>20000</td>
<td>99</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>300</td>
<td>610</td>
<td>630</td>
<td>4</td>
<td>20000</td>
<td>99</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>350</td>
<td>710</td>
<td>730</td>
<td>4</td>
<td>20000</td>
<td>99</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>400</td>
<td>810</td>
<td>830</td>
<td>4</td>
<td>20000</td>
<td>99</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>250</td>
<td>510</td>
<td>530</td>
<td>4</td>
<td>20000</td>
<td>99</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Pulsed ESRM - Specifications

- Frequency of operation: 6-18 GHz and 33-37 GHz
- Temperature range: 4.2K to 310 K at 17 GHz, and ~10-40 ºC at 35 GHz
- Environmental conditions: Normal atmosphere, N₂, He or sealed samples.
- Spin sensitivity: ~10^7 spins for 17 GHz RT; ~10^6 spins for 35 GHz RT and ~10^5 spins for 17 GHz at 4.2 K.
- Gradient magnitude: ~ 200 T/m at 17 GHz, ~ 500 T/m at 35 GHz, by current pulses of 150 ns and 9 A, up to 1400 ns and 94 A.
- Image resolution:
  - Solid samples: ~ 1 micron at 17 GHz RT; ~ 400 nm at 35 GHz RT; experiments at 4.2 K are still on-going.
  - Liquid samples: ~ 10 microns at 17 GHz, RT; ~5 microns at 35 GHz, RT.
- Max image size: ~2000×2000×256 voxels
- Repetition rate: up to 40 kHz (20 kHz in the larger gradients set)
- Modes of image acquisition: 1D, 2D, and 3D (4D is being developed). Phase gradients in X and Y with frequency or phase gradient in Z.
ESR Microscopy – systems architecture and specifications: CW ESRM
Control software
### FFL parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFL Modulation amplitude (peak to peak [0..1]:</td>
<td>0.4</td>
</tr>
<tr>
<td>Field scan (volts [0..4])</td>
<td>4</td>
</tr>
<tr>
<td>FFL Spectrum points</td>
<td>64</td>
</tr>
<tr>
<td>FFL Conversion Factor [nsec]</td>
<td>20</td>
</tr>
</tbody>
</table>

### Coils parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coils resistance [Ohm]</td>
<td>1.5</td>
</tr>
<tr>
<td>Coils response [Tesla] / [Meter * Amp]</td>
<td>2.8</td>
</tr>
<tr>
<td>Gradient Driver response [Amp / Volt]</td>
<td>0.2</td>
</tr>
<tr>
<td>Maximum mean power on the coils [Watt]</td>
<td>2</td>
</tr>
</tbody>
</table>

### Lock-in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFL Time Constant</td>
<td>10 ms</td>
</tr>
<tr>
<td>POST Time Constant</td>
<td>none</td>
</tr>
<tr>
<td>Bandpass</td>
<td>OUT</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>1 mY</td>
</tr>
<tr>
<td>Dynamic Reserve</td>
<td>NORM</td>
</tr>
<tr>
<td>Reference mode</td>
<td>off</td>
</tr>
</tbody>
</table>

### Lock-in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFL Time Constant</td>
<td>300 ms</td>
</tr>
<tr>
<td>POST Time Constant</td>
<td>none</td>
</tr>
<tr>
<td>Bandpass</td>
<td>OUT</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>300 mY</td>
</tr>
<tr>
<td>Dynamic Reserve</td>
<td>NORM</td>
</tr>
<tr>
<td>Reference mode</td>
<td>off</td>
</tr>
</tbody>
</table>
CW ESRM - Specifications

- Frequency of operation: 6-18 GHz (either with a commercial bridge or a “home-made” YIG tuned system)
- Temperature range: ~10-40 °C
- Environmental conditions: Normal atmosphere, N₂, He or sealed samples.
- Spin sensitivity: being examined... (~10⁷-10⁸ spins)
- Max gradient magnitude: ~ 7.5 T/m with currents of up to 3A.
- Image resolution:
  - Solid samples: ~ 10 micron at 17 GHz.
  - Liquid samples: being examined...
- Max image size: ~256×256×256×64 voxels
- Modes of image acquisition: Spatial-spatial and spatial spectral 1D, 2D, 3D and 4D.
ESR Microscopy – Examples for sensitivity and imaging capabilities

Pulsed ESRM – room temperature

35 GHz, solids and liquids
LiPc Crystal, #1, pure 2D image

- Image size: $1800 \times 260$ pixels
- $\tau = 800$ ns
- Acquisition time – 2.5 h
- Rep rate – 6 kHz
- Resolution: $\sim 440 \times 650$ nm
- Spin sensitivity of $\sim 1.8 \times 10^7$ spins
LiPc Crystal, #1, 2D slices from a 3D image

- Image size: 256×50×20 voxels
- τ=800 ns
- Acquisition time – 25 min
- Rep rate – 10 kHz
- Resolution: ~2.2×3.25×3.5 μm
- spin sensitivity of ~6.2×10^7 spins
LiPc Crystal, #2, 2D slices from a 3D image

- 160×100×170 voxels
- \( \tau = 800 \) ns
- 5 hours
- Rep rate – 10 kHz
- Resolution: \( \sim 1.1 \times 1.62 \times 1.75 \) \( \mu m \)
- Spin sensitivity of \( \sim 10^7 \) spins
1mM trityl solution around a nylon mesh, 2D slice from a 3D image

- Image size: 128×64×32 and 220×100×32 voxels
- \( \tau = 300 \text{ and } 360 \text{ ns} \)
- Acquisition time – 75 min to 5 h
- Rep rate – 30 kHz
- Resolution: \( \sim 10.5 \times 11.7 \times 40 \text{ and } \sim 6.8 \times 7.7 \times 40 \text{ \( \mu \text{m} \)} \)
- Spin sensitivity of \( \sim 8 \times 10^7 \text{ and } 3 \times 10^7 \text{ spins} \)
Initial 5K imaging results – N@C₆₀ sample, 2D image, ~6 μm resolution
Initial 5K imaging results – polycrystalline Si layer 1 μm thick, 2D image, ~110 μm resolution
Biological applications

- Oxygen concentration near cells*

$T_2$ vs. $[O_2]$
**$T_2$ Imaging of Trityl in Glycerol/water mixtures**

(a) Image showing four chambers with different glycerol/water mixtures: 20%, 35%, 50%, and 60%, each labeled with their respective percentage. The scale bar indicates 500 μm.

(b) Color maps representing the $T_2$ imaging of the four chambers. The color scale ranges from 0.3 to 1.0.

(c) Magnified views of the color maps showing the spatial distribution of $T_2$ values at different locations.

(d) Histograms showing the pixel occurrence of $T_2$ values for each chamber, with $T_2$ values ranging from 900 to 1500 ns.
T₂ and O₂ imaging near live cells

Cyanobacteria

350μm
Raw data images of ESR signal

**Dark**
- $\tau = 500$ ns
- $\tau = 600$ ns
- $\tau = 700$ ns

**Light**
- $\tau = 500$ ns
- $\tau = 600$ ns
- $\tau = 700$ ns

90° 180° echo

MW

$G_x \sim 4$ T/m

$G_y \sim 4$ T/m

$G_z \sim 0.8$ T/m
T$_2$ and O$_2$ imaging near live cells

Radical concentration

T$_2$/O$_2$ images

Dark

Light

Figure legend:
- Z=0
- Z=100 μm
- Z=200 μm

T$_2$ [ns] O$_2$ [μM]

Dark:
- T$_2$: 250, 320, 390, 460, 530, 600 ns
- O$_2$: 185, 218, 262, 322, 407, 540 μM

Light:
- T$_2$: 250, 320, 390, 460, 530, 600 ns
- O$_2$: 185, 218, 262, 322, 407, 540 μM
O₂ diffusion simulation

![Diagram](image)

- **Air outlet to the atmosphere**: 200 μm
- **O₂-producing “sample”**

[μmol/l]

- 500
- 450
- 400
- 350
- 300
- 250
- 200
Biological applications

- Superoxide in roots - Arabidopsis thaliana
Arabidopsis thaliana

Typical 6 days old plant in agar
Radicals employed in the roots study

PTM-TC

PD-TEMPO, $^{15}\text{N}$
PTM-TC signal in an intact plant as a function of time

- ESR signal of whole plant measured with a Bruker system and a Bruker rectangle resonator.
- During 30 minutes there no apparent change in plant signal, vs the reference signal.
- Spin concentration in the plant is ~ 1mM.
- Center field 3299 G, mod. Amp. 1.3 G.
PTM-TC signal in an injured plant as a function of time

- Typical ESR signal of the entire plant after injury made in the leafs (Bruker resonator)
PTM-TC signal in several injured plants as a function of time

- Collection of kinetic plots for WT and RBOHD mutants for entire plants after injury (with Bruker resonator)
Special dielectric resonator for 1D ESRM
Special dielectric resonator for 1D ESRM
Special plant holder for 1D ESRM

- Image of the set-up for the plant holder for the new DR (photo taken through a macro lens)
Special plant holder for 1D ESRM

- Close-up of the plant in the sample holder, photograph through a microscope
Spin sensitivity: DR vs. rectangular resonator

- ESR signal in Bruker and new dielectric resonator (DR) for capillary tube, 0.5 mm OD filled with 1mM PTM-TC in water.
- The DR is ~13.2 more sensitive per unit of length.
• 1D image of a capillary tube with PTM-TC in water, gradient current 0.6A provides gradient of ~ 0.25 T/m.

• Raw resolution is given by: $\Delta z \approx \frac{2\Delta B_{1/2}}{G_z}$, which gives ~ 1 mm, but deconvolution improves it by a factor of ~ 5 to ~ 200 μm.
PTM-TC signal in an injured plant as a function of time: distal part of the root in the DR

Whole distal ~4 mm of the root
Biological applications

- Intracellular imaging: \([O_2]\) in nematodes
A single nematode on the sample holder
Nematode 2D slices of a 3D image

- Image size: 64×32×100 voxels
- $\tau=300$ ns
- Acquisition time – 1 h
- Rep rate – 30 kHz
- Resolution: $\sim18\times18\times40$ μm
- Spin sensitivity of $\sim5\times10^7$ spins
Biological applications

➢ Tissue imaging: \([O_2]\) in cancer spheroids
Future directions

- Continued improvements in sample preparation methods and developments of spin probes for biological applications
- Sensitivity and resolution improvements
  - Increased rep. rate
  - Application specific probes
  - Pre-polarization pulse?
  - 60 GHz probe?
  - Measurements of fixed samples
  - Improved radicals?
- Combination of ESRM with diffusion measurements (PGSE-ESR*)
- Surface and superconducting resonators
- “Exotic” structural biology applications

*Phys. Chem. Chem. Phys., 2010, 12, 5998-6007*
Surface and superconducting resonators

New Micro-loop gap surface resonators

Microwave magnetic field at the center of the resonator

- Purple: Rutile
- Blue: LGR150
- Red: LGR50
- Green: Omega

Distance above resonator surface [microns] vs. $H_1 [\text{A/m}]$
Comparison of ESR signal from various resonators

- Current surface resonator has room temp. sensitivity of ~10^5 spins per 1 h of acquisition.
- Replacing the gold by superconducting material may provide at low temp single spin sensitivity.
- Combination of powerful gradients with single spin sensitivity can result in a variety of applications ranging from basic physics, semiconductors, to structural biology.
Summary

- ESRM has progressed to a level of practical applications
- Specific methodological tools can be developed to tackle specific important problems
- Advances in spin probes would greatly expand the scope of ESRM
- Additional methodological advances are being pursued.
Acknowledgments

- Current and past group members working on the presented topics
  - Revital Halevy
  - Michael Shklyar
  - Dr. Lazar Shtriberg
  - Vladislav Rinsky
  - Yael Talmon
  - Alon Platner
  - Nasim Warwar
  - Omri Arbiv
  - Alex Katckis
  - Rami Maymon
  - Katerina Suhavoy
  - Dr. Ygal Twig

**Collaborators:**
- Wolfgang Harneit - FU Berlin (N@C_{60})
- Klaus Lips – Helmholtz-Zentrum Berlin (Si)
- Rober Flhour – Weizmann (Roots)
- Periannan Kuppusamy – OSU (PTM-TC)
- Victor Tormyshev - Novosibirsk (deuterated trityl)
- Jack Freed

http://mr-lab.technion.ac.il/

- Israel Science Foundation
- US-Israel Binational Science Foundation
- European Research Council
- German-Israel Science Foundation