Porphyrin-phospholipid Liposomes

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Brief Background

- Composed of a lipid Bi-layer
- Used to improve bio-distribution and efficacy of anti-cancer agents via reduced systematic toxicity
- Currently 12 drugs are FDA approved to be delivered liposomally, with several in trials
- History in Buffalo

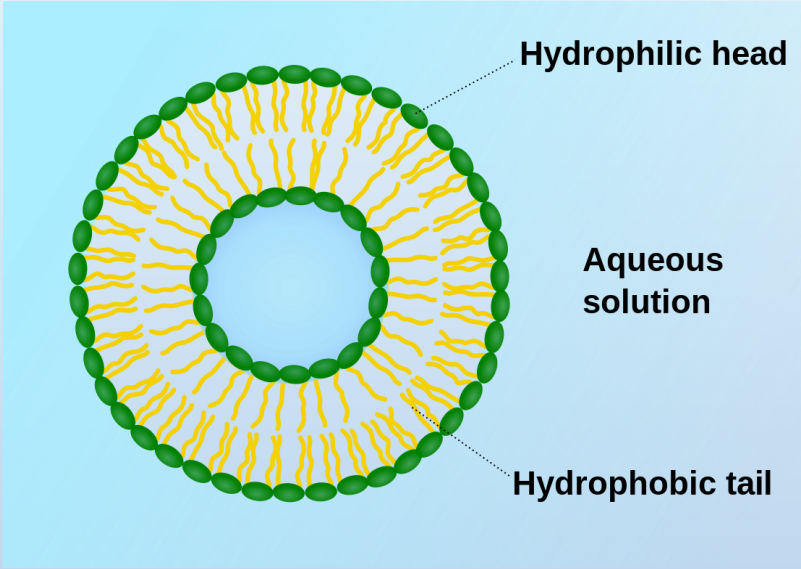

Figure 1

Recent Research

- Much research has been focused on triggering localized drug release via external stimuli
- Several light-triggered release mechanisms have been looked at

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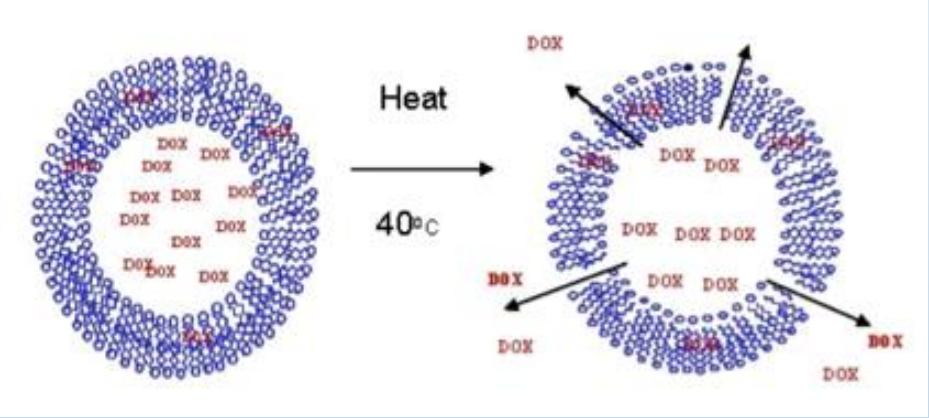

Figure 2

Lovell Lab

nature COMMUNICATIONS

ARTICLE

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Porphyrin–phospholipid liposomes permeabilized by near-infrared light

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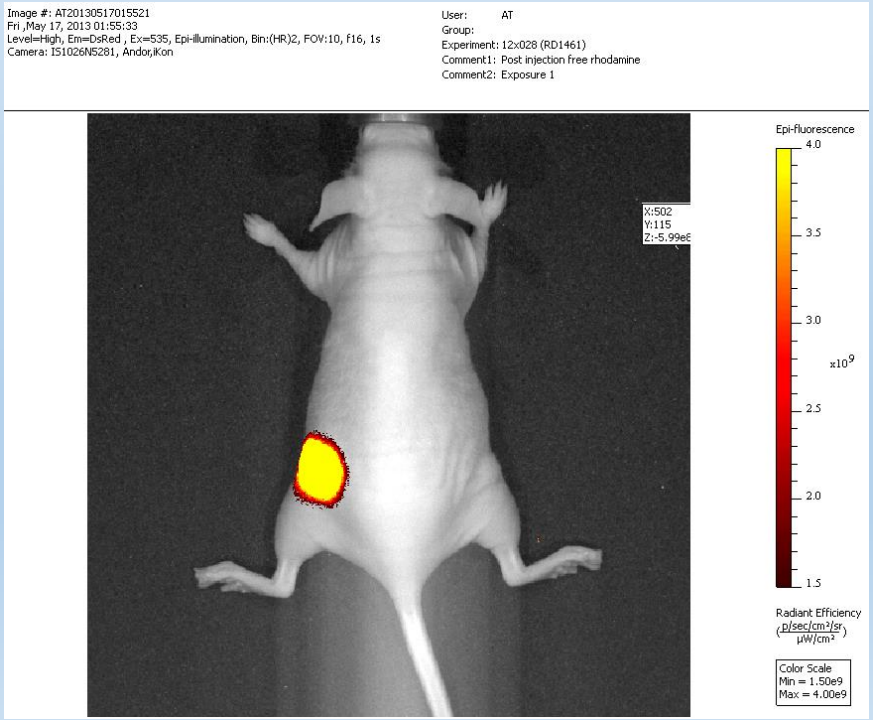
- Incorporating the photosensitizer HPPH into the liposome formulation, our lab found that irradiation of the formed nano-particles with a wavelength corresponding to the peak of HPPH induced permeabilization of the liposomes



Focus of my Research

- Up until recently, characterization of the bio-distribution of liposomes has been performed via fluorescence imaging of both HPPH and Doxorubicin, a commercial and potent chemotherapeutic drug delivered liposomaly
- To better confirm the distribution and release kinetics of the PoP-liposomes, Gadopentatic acid (Gd-DTPA) was actively loaded into the particles, and the release can be measured through T1 weighted MR scans

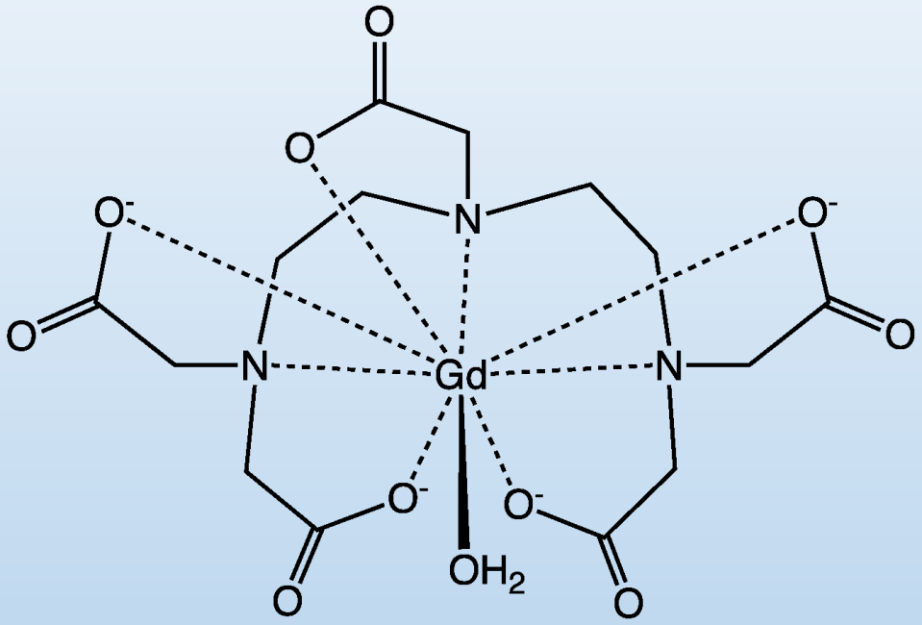
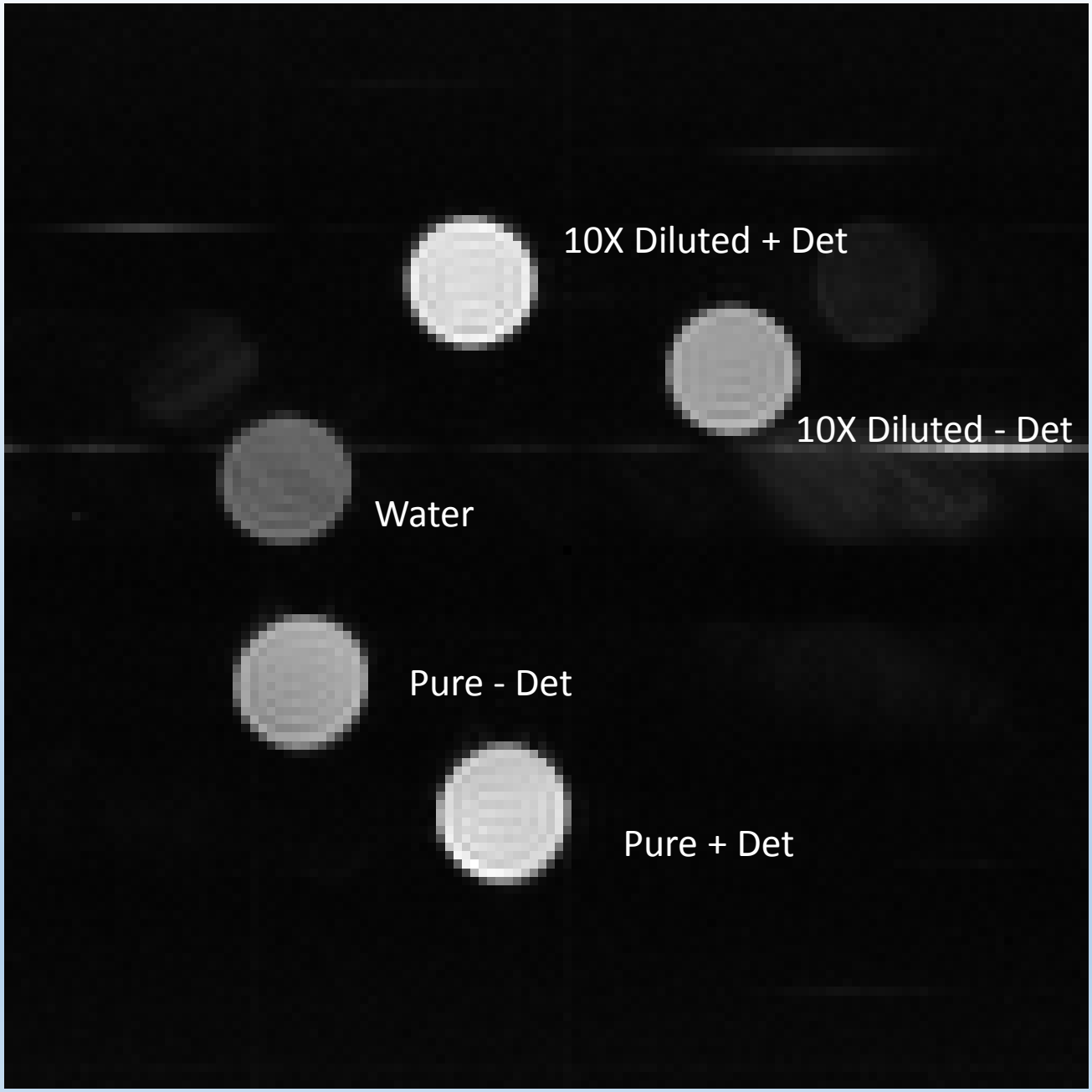
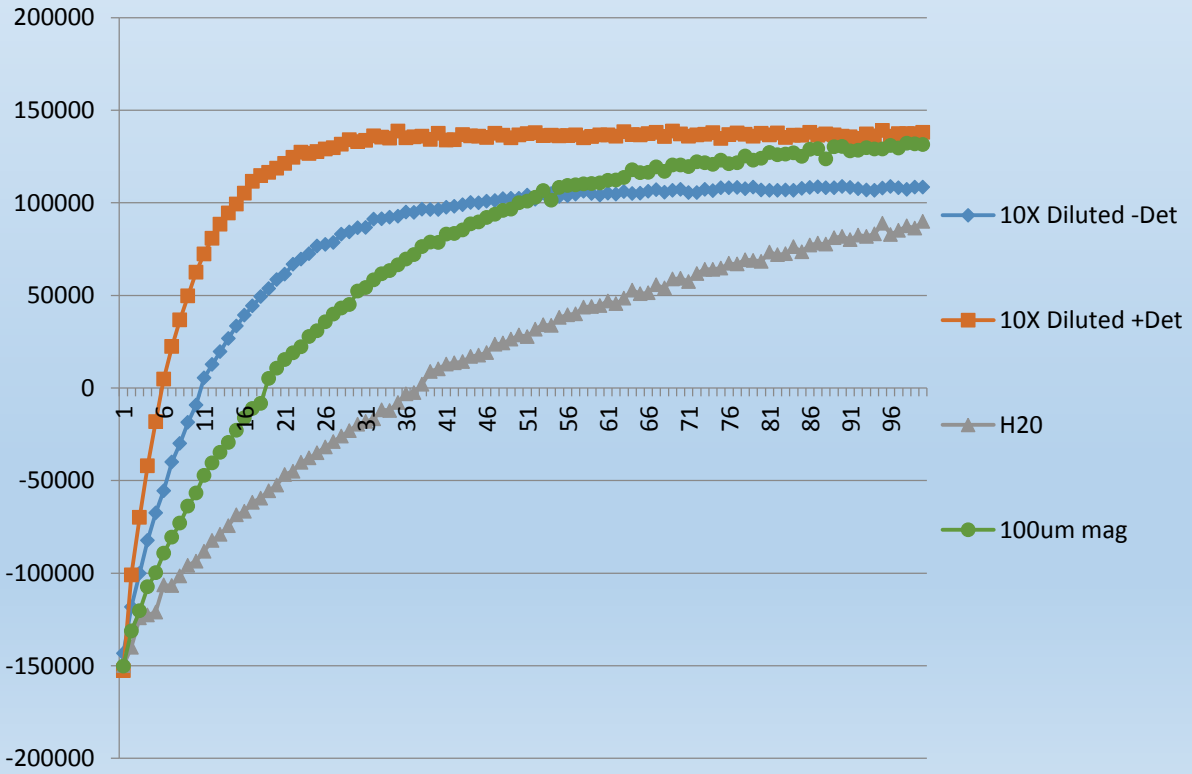


Figure 3


10X Diluted + Det
10X Diluted - Det
Water
Pure - Det
Pure + Det

Sample Relaxation Rates

Sample	Water	Pure Sample	Lysed Pure Sample	10X Diluted	Lysed 10X
Relaxation Rate	0.45231	12.11740	20.00000	1.77990	3.09910
Goodness of Fit	0.9988	0.9972	0.9509	0.9996	0.9992



Pilot Study 1

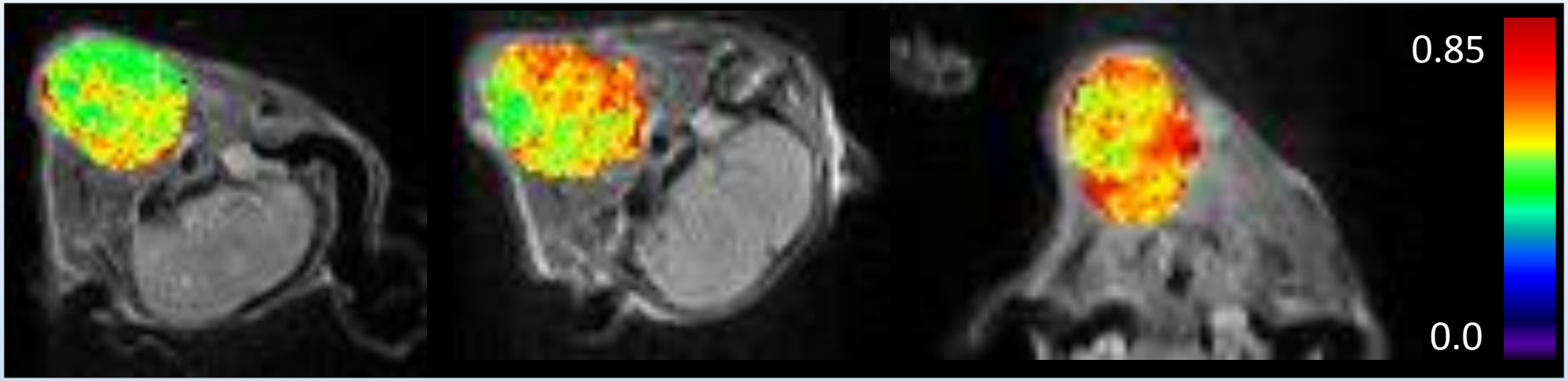
- IV injection of Gd PoP-Liposomes
- MRI (baseline, 1hr, 24hr, 72hr)
- BLI/Fluorescence (24hr, 72hr)

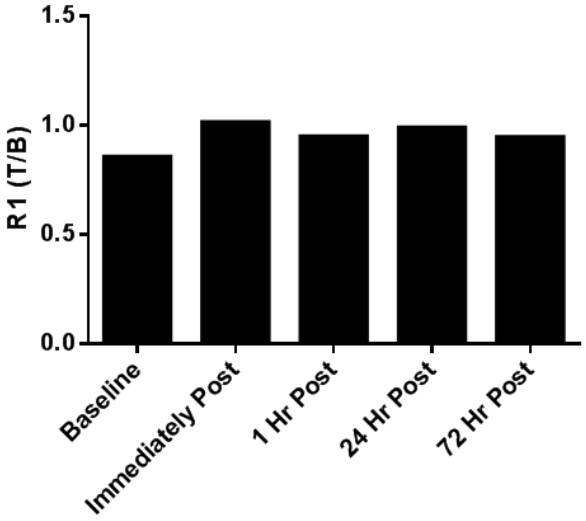
Pre-PoP

Immediately post

24 hour post

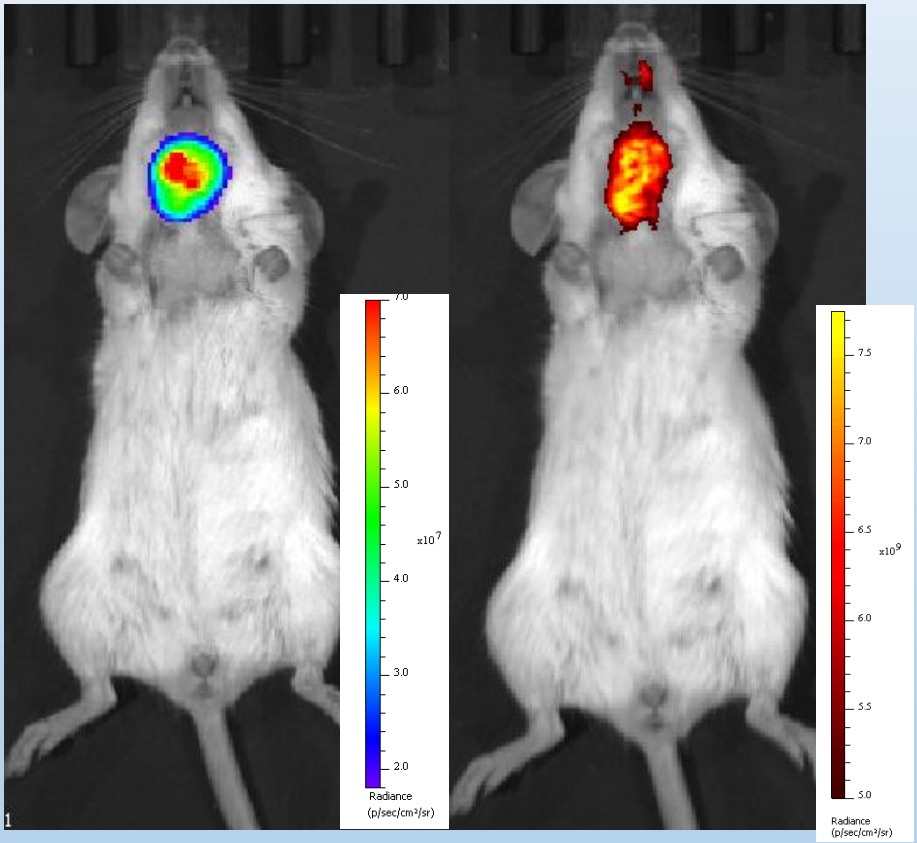
ctrl





24 Hours Post

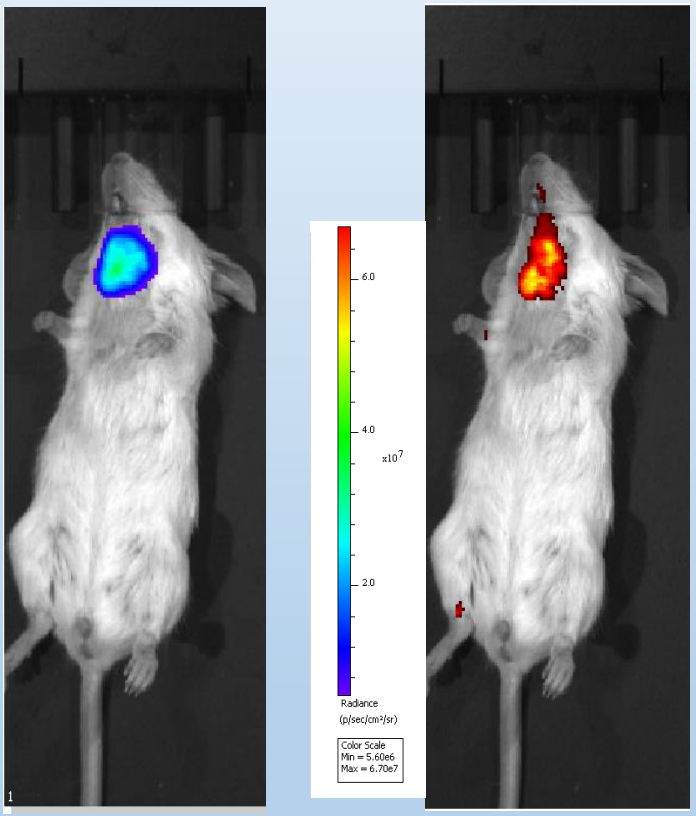
mD3 (control)



BLI Fluorescence

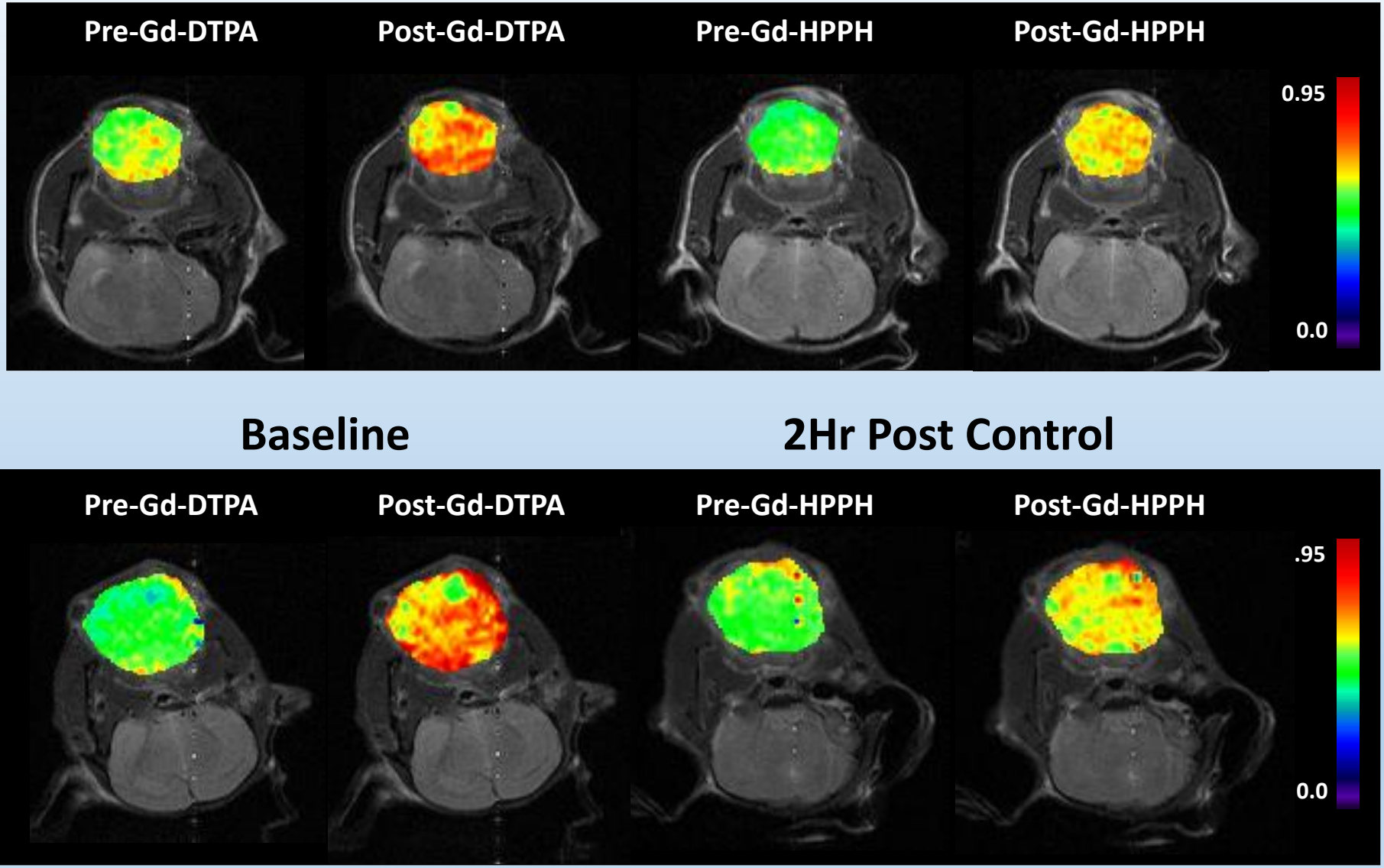
72 Hours Post

mD3 (control)

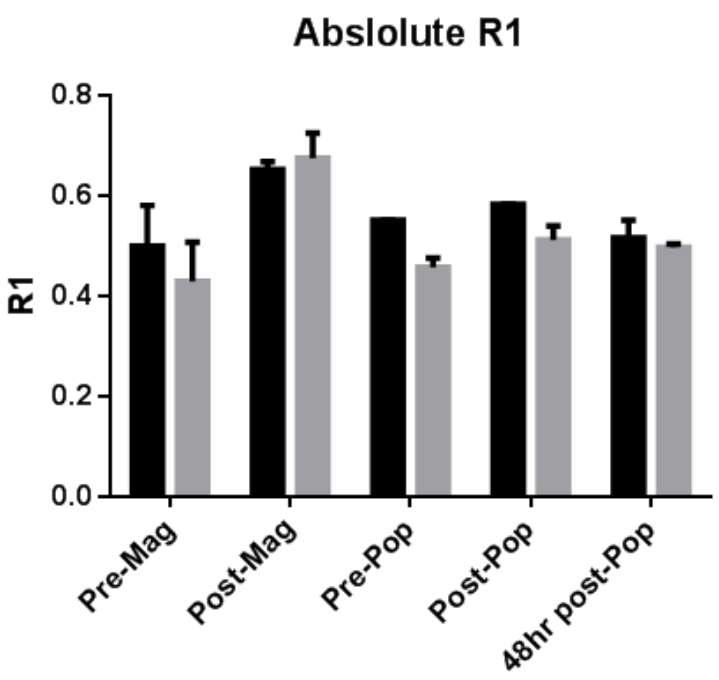


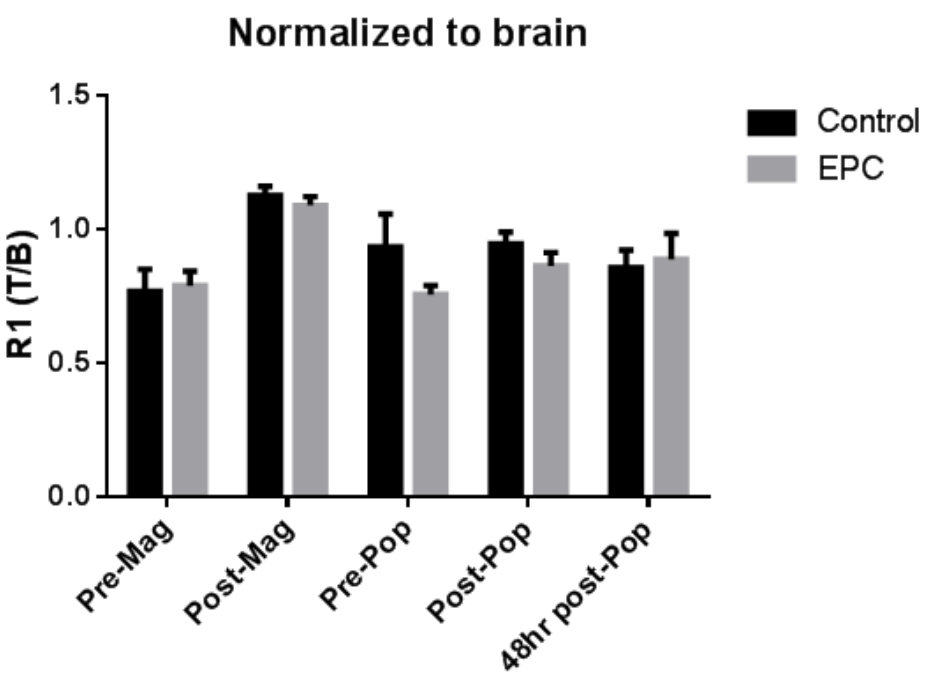
Pilot Study 2

- IV injection Gd-DTPA (baseline) followed by HPPH-Gd PoP (2hr post EPC)
- 2 control, 2 EPC (2hr prior to HPPH-Gd)
- MRI (baseline, 2hr post EPC (baseline PoP), 4hr, 48hr)
- BLI/Fluorescence (2hr post Epc (baseline PoP), 4hr post, 24hr post, 48hr post)

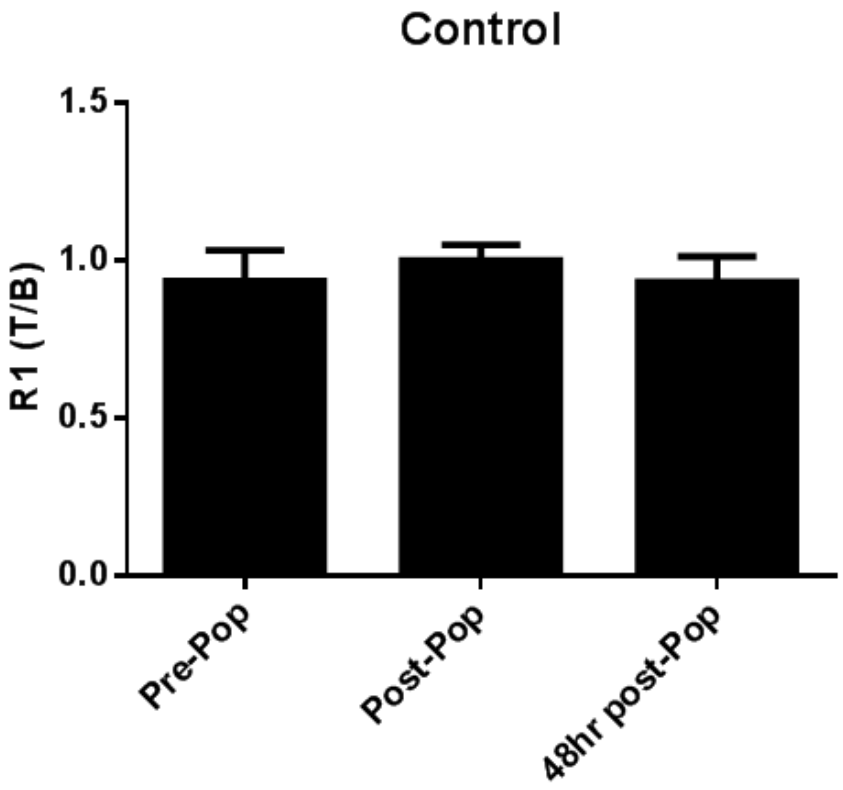

Baseline
2Hr Post-EPC
Pre-Gd-DTPA
Post-Gd-DTPA
Pre-Gd-HPPH
Post-Gd-HPPH
0.95
0.0
Baseline
2Hr Post Control
Pre-Gd-DTPA
Post-Gd-DTPA
Pre-Gd-HPPH
Post-Gd-HPPH
.95
0.0

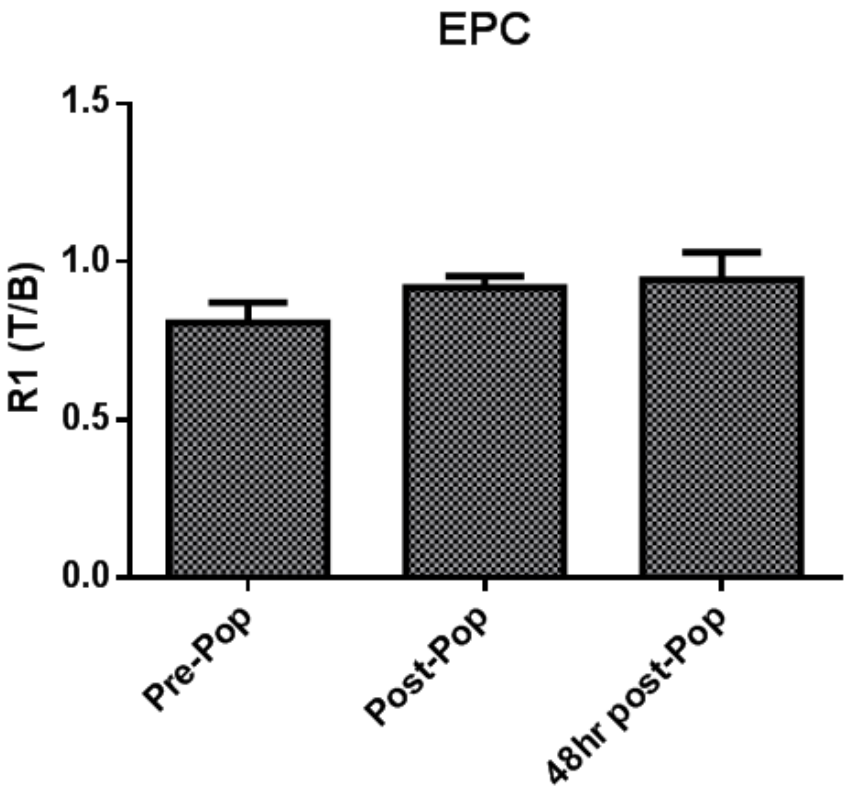
R1 values


Abslolute R1
R1
0.8
0.6
0.4
0.2
0.0
Pre-Mag
Post-Mag
Pre-Pop
Post-Pop
48hr post-Pop


Normalized to brain
R1 (T/B)
1.5
1.0
0.5
0.0
Pre-Mag
Post-Mag
Pre-Pop
Post-Pop
48hr post-Pop
Control
EPC

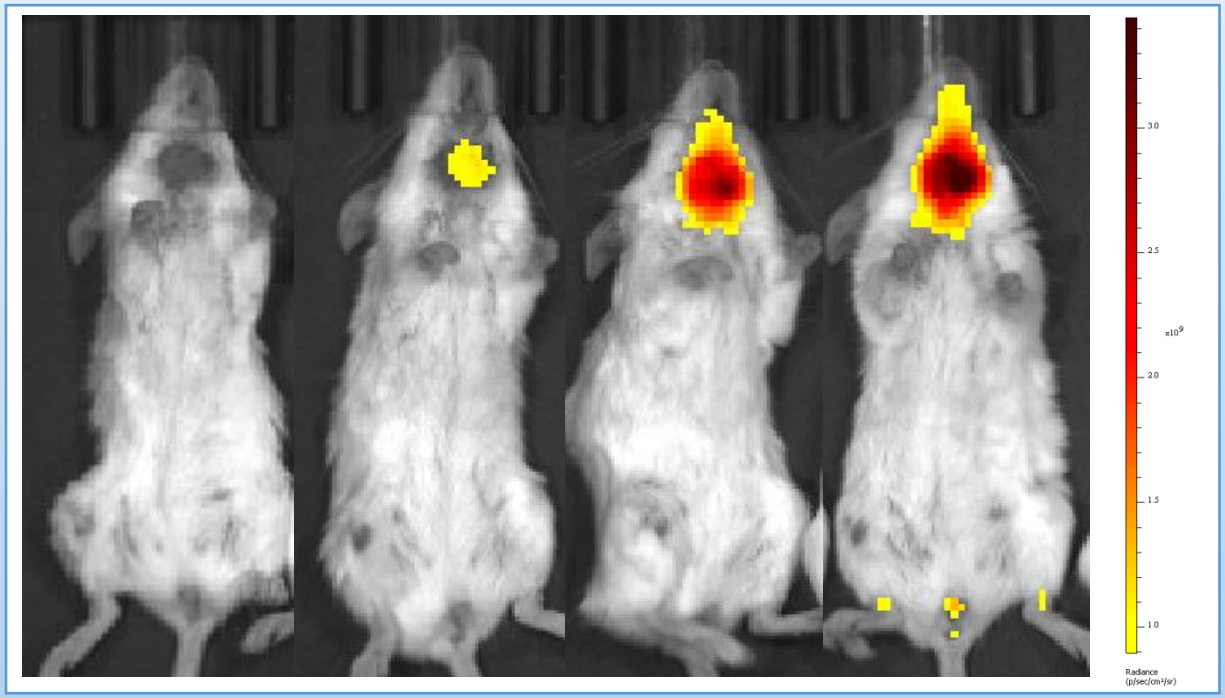
Enhancement From PoP





- It appears that the EPC group sustains enhancement while the control group goes back to baseline levels at 4hrs post PoP.

HPPH-Gd Fluorescence Time Course



- Although the MRI data suggests a reduction in the R1 starting at 4hrs, this control shows an accumulation of the HPPH-Gd beginning at 4hrs and increasing up to 48hrs.

Pilot Study 3

- Intra-Tumoral injection HPPH-Gd PoP
- 1 control
- MRI (pre/post injection)
  - VTR, 3D-SPGR
- Ultra-Sound guided injection

Us guided Injection

Pre-PoP IT

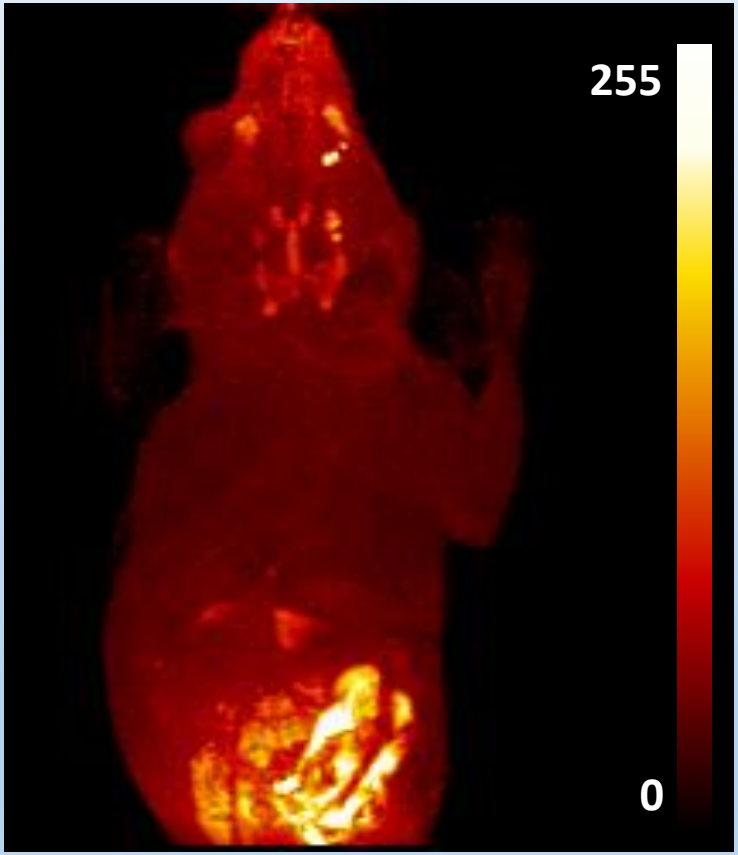
Clear image of needle

Post-PoP IT

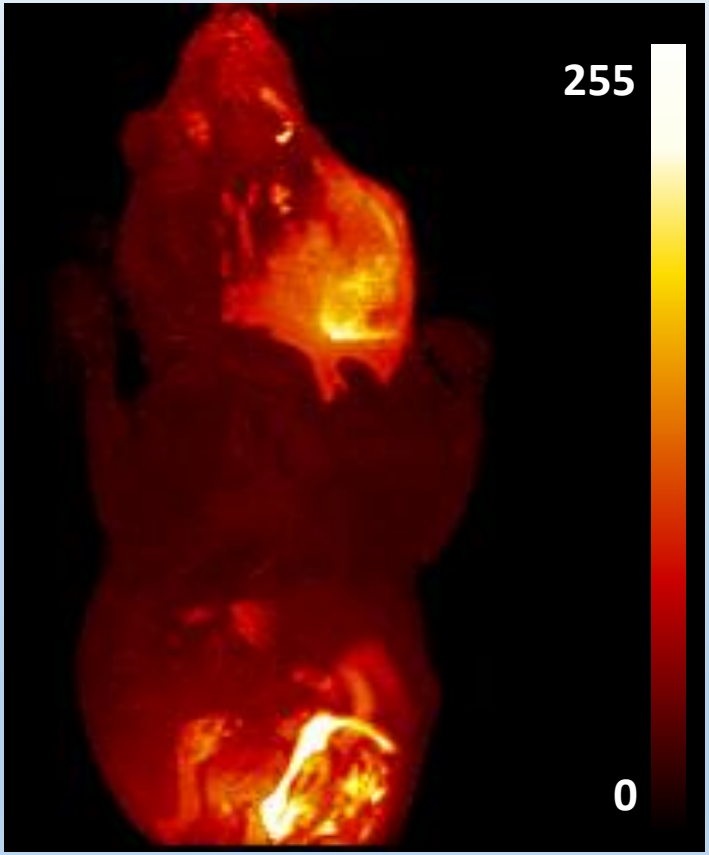
- Using ultrasound guided injection, it was possible to inject into center of the tumor.
- R1 map on the right shows clear enhancement in the center of the tumor, likely where bulk of HPPH-Gd was injected
- However, some did leak and into the sub-Q space

HPPH-Gd MIP

Pre-PoP IT

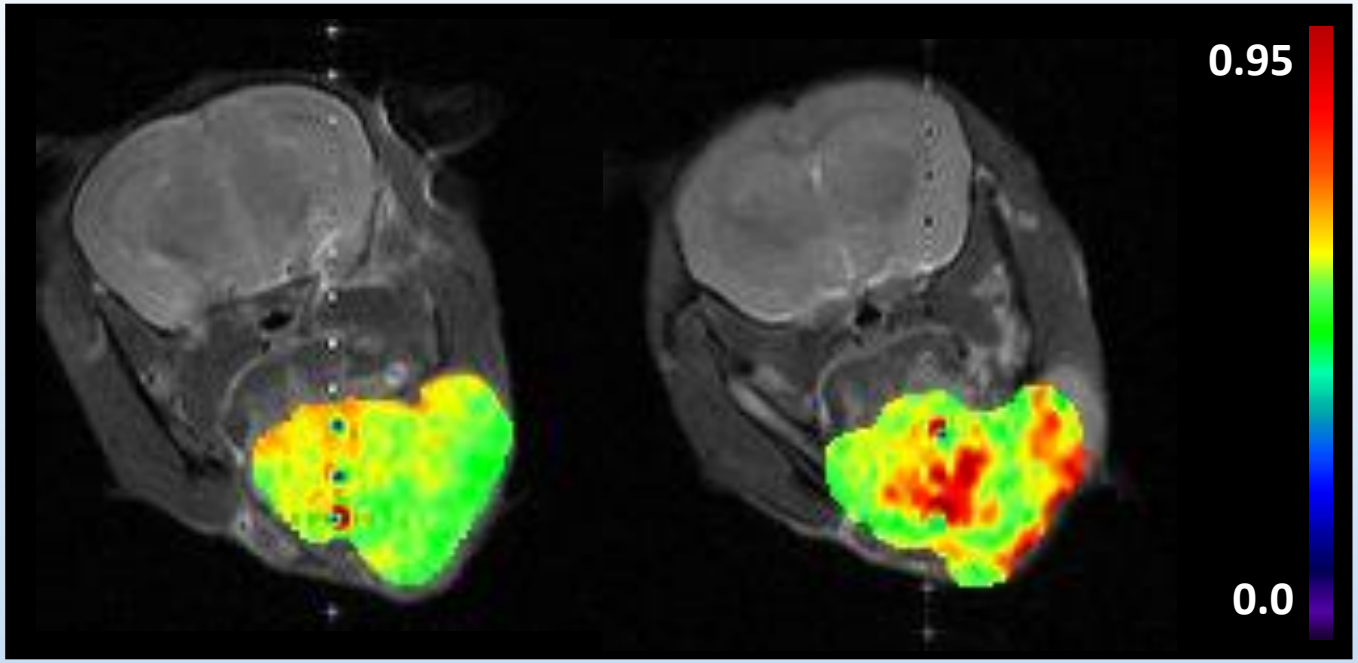


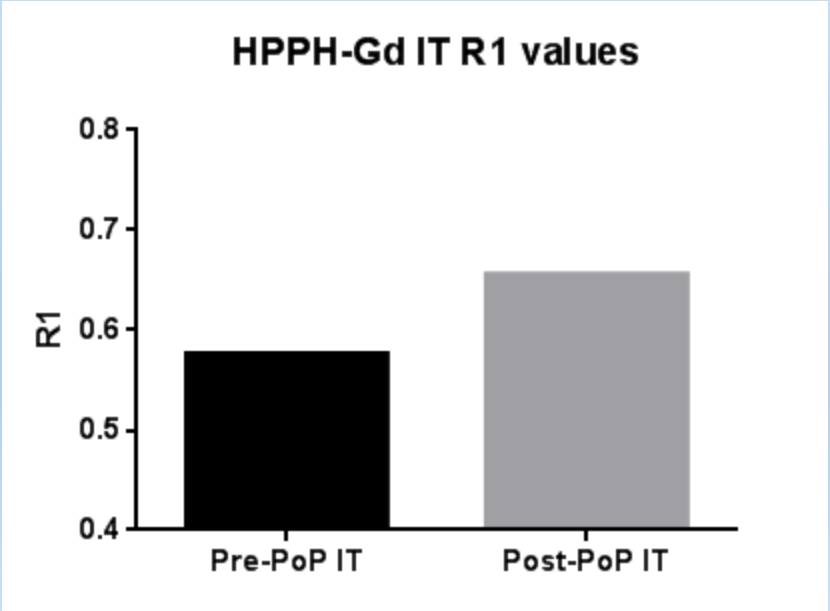
Post-PoP IT



Pre-PoP IT

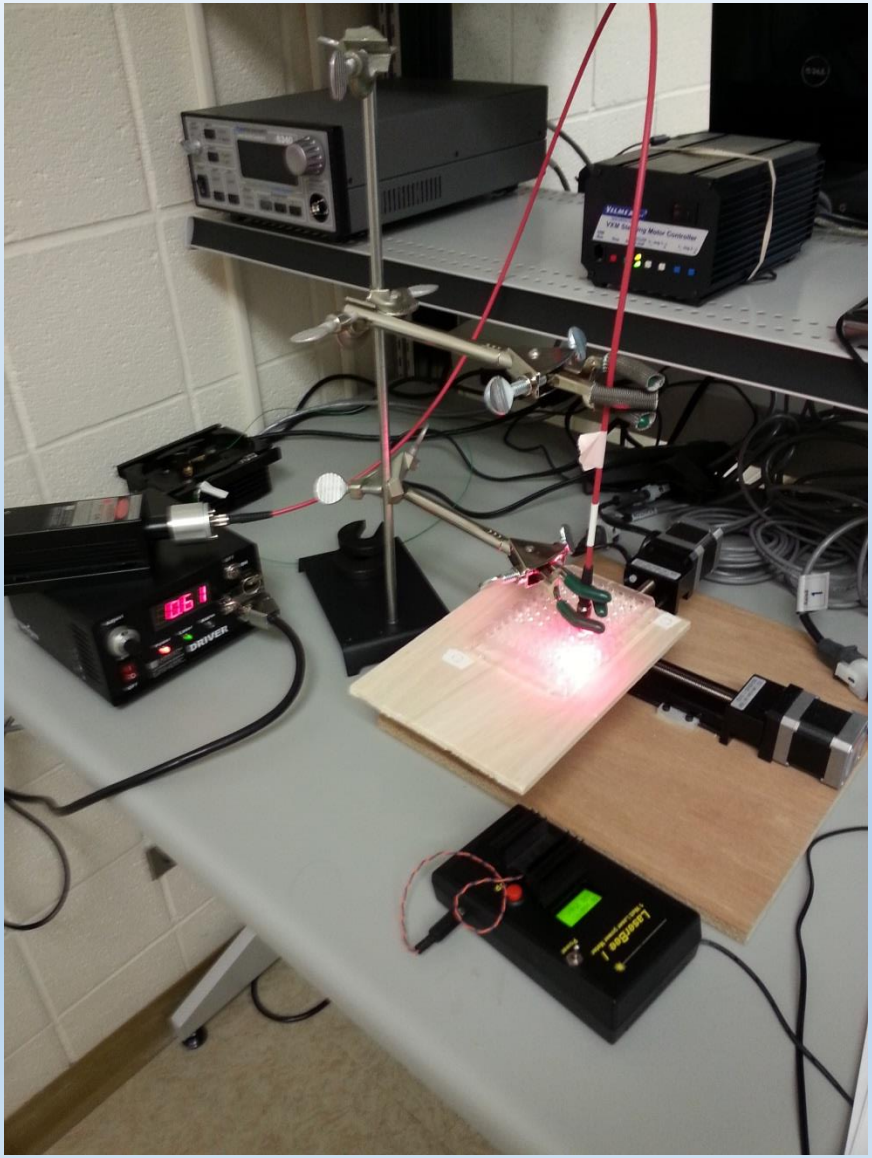
Post-PoP IT

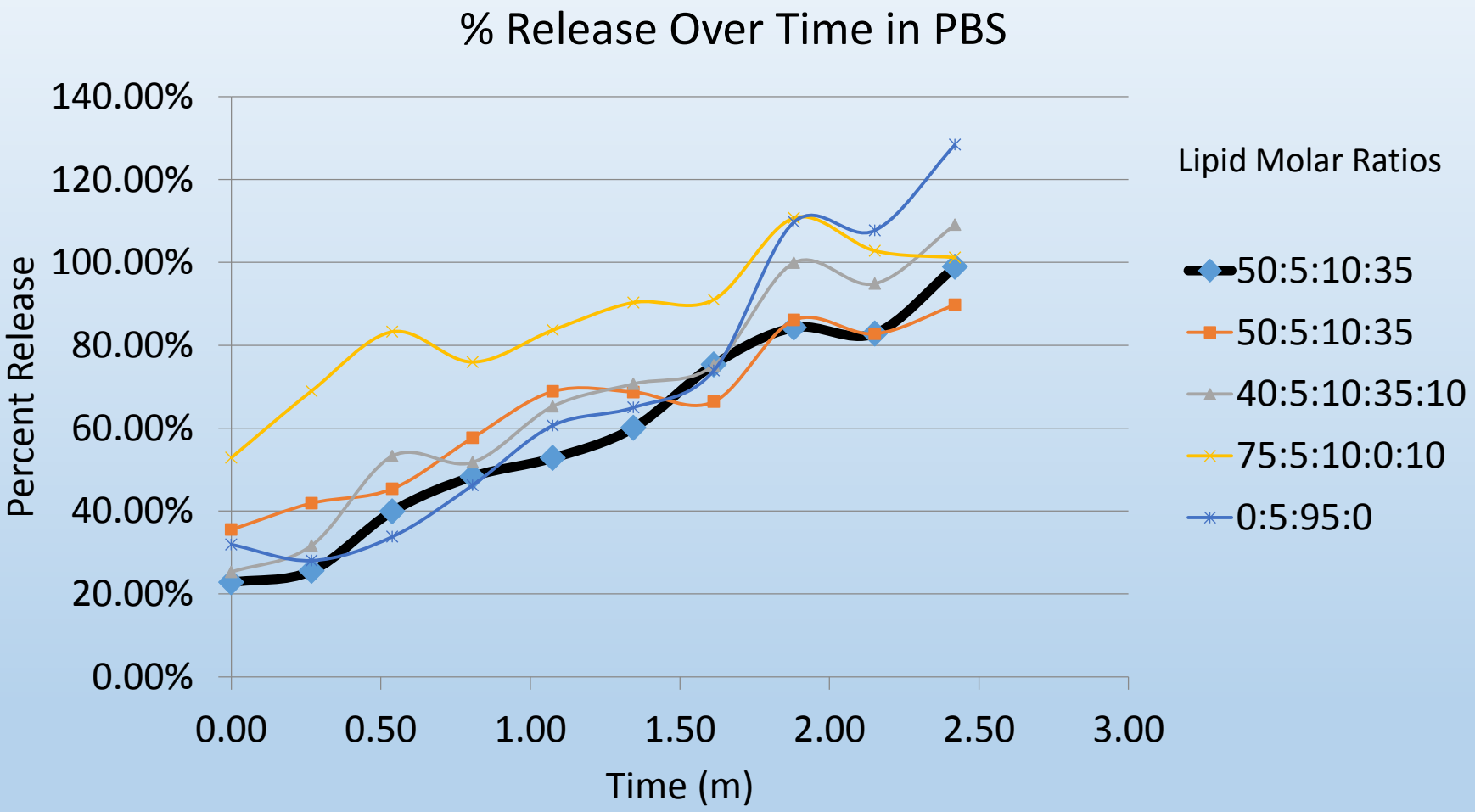


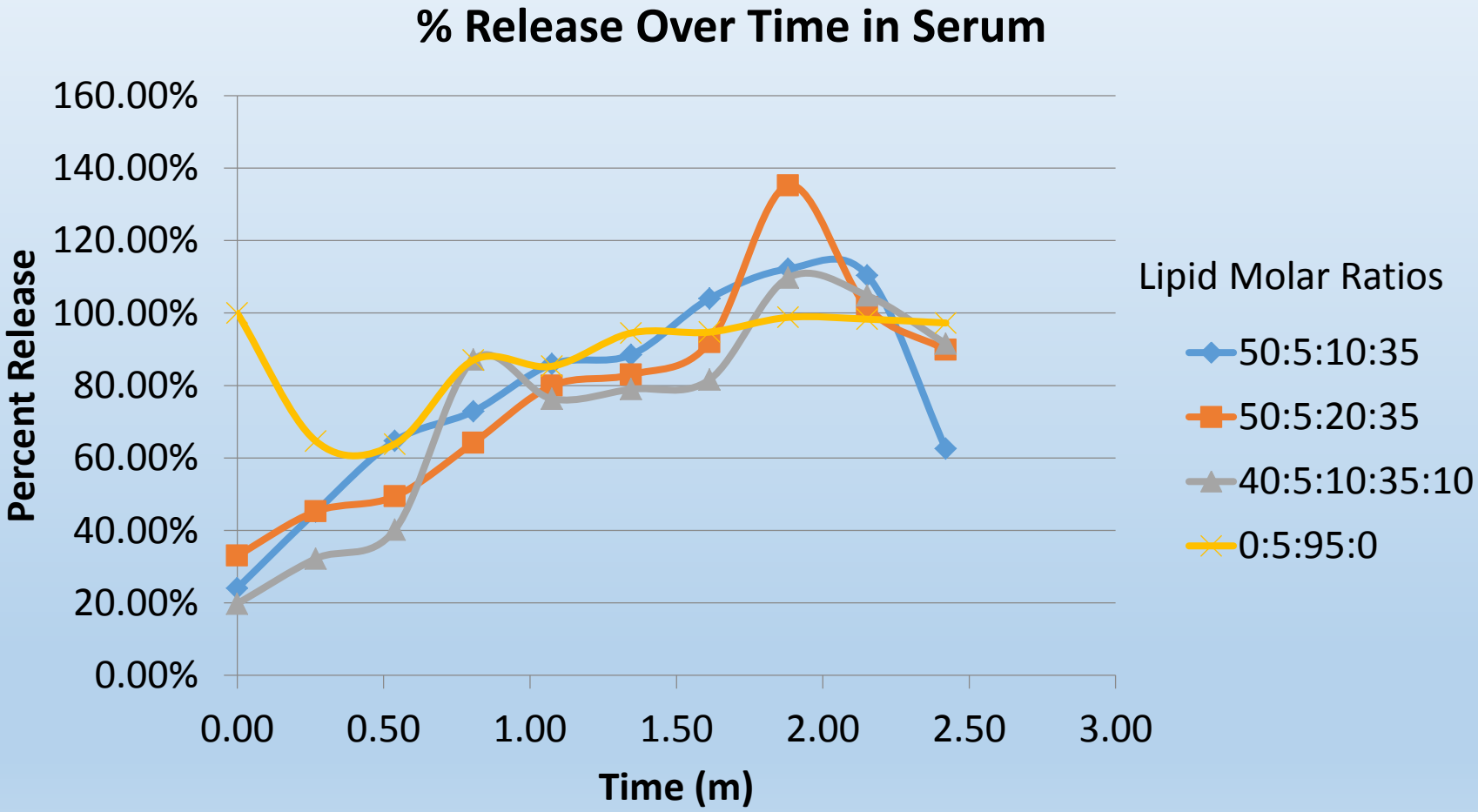


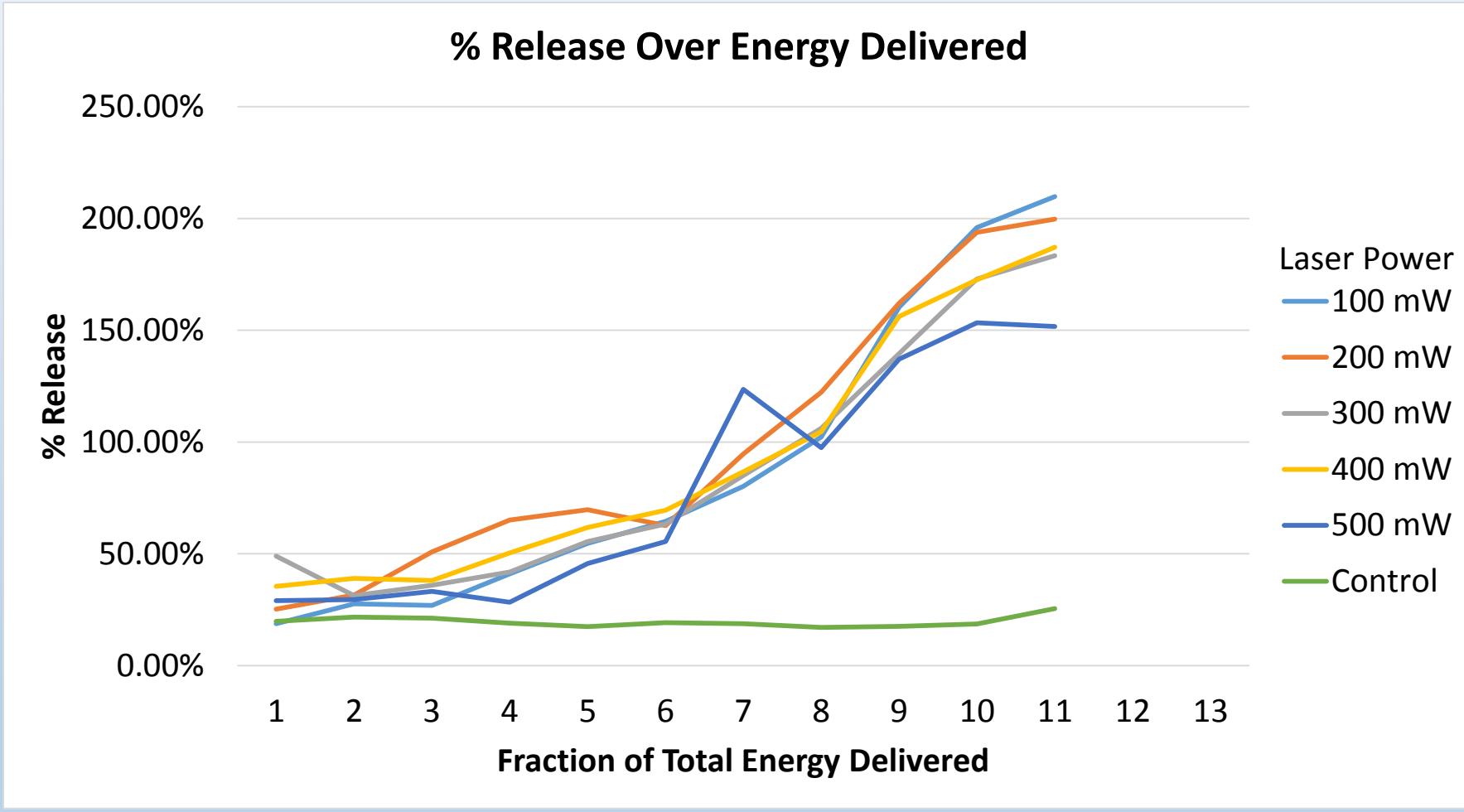
Other Work with PoP-Liposomes

- Looking at release kinetics of liposomes with varying molar ratios of lipids
- Monitoring release as a function of power delivered at varying fluence rates




% Release Over Time in PBS
140.00%
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Percent Release
0.00
0.50
1.00
1.50
2.00
2.50
3.00
Time (m)
Lipid Molar Ratios
50:5:10:35
50:5:10:35
40:5:10:35:10
75:5:10:0:10
0:5:95:0


% Release Over Time in Serum
Percent Release
160.00%
140.00%
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
0.00
0.50
1.00
1.50
2.00
2.50
3.00
Time (m)
Lipid Molar Ratios
50:5:10:35
50:5:20:35
40:5:10:35:10
0:5:95:0


% Release Over Energy Delivered
250.00%
200.00%
150.00%
100.00%
50.00%
0.00%
% Release
1
2
3
4
5
6
7
8
9
10
11
12
13
Fraction of Total Energy Delivered
Laser Power
100 mW
200 mW
300 mW
400 mW
500 mW
Control

Future Plans

- Check the feasibility of light release in serum of Gd-DTPA for different time points
- Intra-tumoral injections of sample monitored regularly over a period to see whether or not there is a bio-redistribution
- Trying different lipids in the formulation for faster In-Vivo release
- Exam changes in T2 rates for our Gd-HPPH particles

Enormous thanks to:
Kevin Carter from the Lovell Lab
Laurie Rich from MR lab at RPCI

Everyone else from the Lovell Lab
Dr. Lovell, Dr. Seshandri, Dr. Spernyak

References

Figure 1 "Liposome scheme-en" by SuperManu - Own work. Licensed under Creative Commons Attribution-Share Alike 3.0-2.5-2.0-1.0 via Wikimedia Commons - http://commons.wikimedia.org/wiki/File:Liposome_scheme-en.svg#mediaviewer/File:Liposome_scheme-en.svg

Figure 2 Taken from ThermoDox website: http://celsion.com/docs/technology_overview

Figure 3 "Gd(DTPA)(aq)2-" by Smokefoot - Own work. Licensed under Creative Commons Attribution-Share Alike 3.0 via Wikimedia Commons - http://commons.wikimedia.org/wiki/File:Gd(DTPA)(aq)2-.png#mediaviewer/File:Gd(DTPA)(aq)2-.png