Neptune

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NEPTUNE

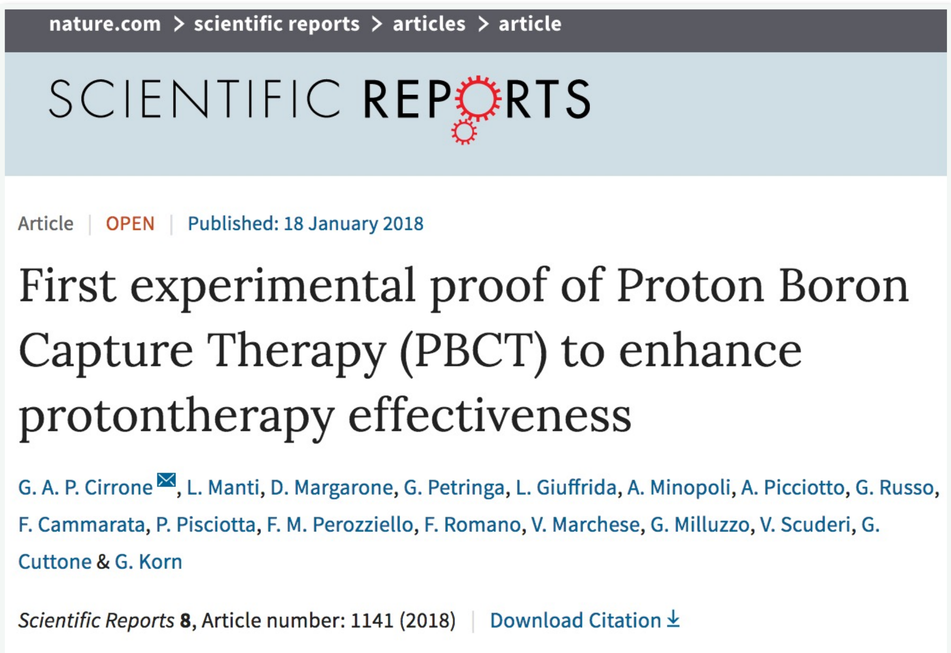
Nuclear process driven Enhancement of Proton Therapy UNravEled

The Neptune project

- Goal: enhancement of proton therapy effectiveness using nuclear reactions
- Technique: administration of borated and fluorinated compounds, that accumulate in tumor, to patients before irradiation

$p + {}^{11}B \longrightarrow 3\ \alpha$ (up to 4 MeV)

$p + {}^{19}F \longrightarrow {}^{16}O + \alpha$ (up to 13 MeV)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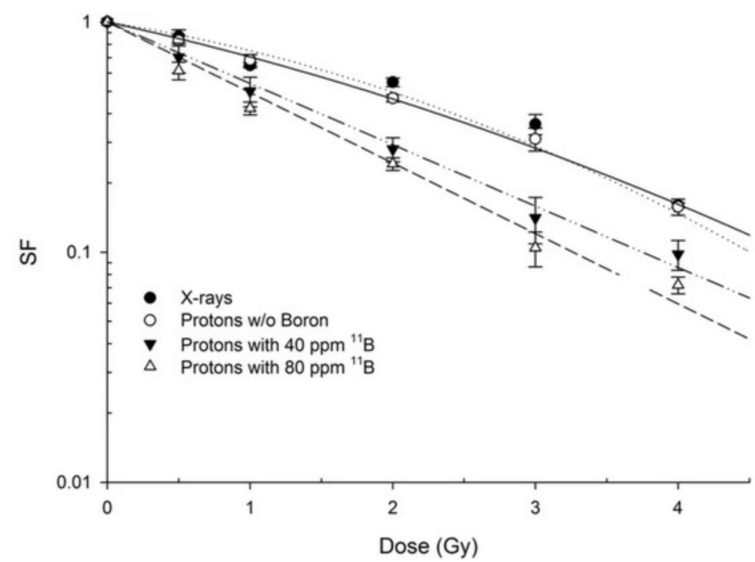
SCIENTIFIC REPORTS

Article | OPEN | Published: 18 January 2018

First experimental proof of Proton Boron Capture Therapy (PBCT) to enhance protontherapy effectiveness

G. A. P. Cirrone, L. Manti, D. Margarone, G. Petringa, L. Giuffrida, A. Minopoli, A. Picciotto, G. Russo, F. Cammarata, P. Pisciotta, F. M. Perozziello, F. Romano, V. Marchese, G. Milluzzo, V. Scuderi, G. Cuttone & G. Korn

Scientific Reports **8**, Article number: 1141 (2018) | Download Citation



Clonogenic Survival Curve @ MID SOBP

The Neptune collaboration

- Activities:
  - modelling of nuclear reactions
  - microdosimetry
  - radiobiology
  - imaging and quantification
- Rome group: **imaging and quantification**

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  A. Messina, D. Rotili
- Other INFN departments: LNL, LNS, MI, NA, PV, Roma3, TIFPA

Neptune status & results

- **Modelling of nuclear reactions**
  - improved simulations are not able to explain the radiobiological effect so far
  => the contribution due to the alpha produced in the nuclear reaction is not sufficient

- **Microdosimetry**
  - experimental campaigns have been performed with 3 different detectors to evaluate the dose at cellular dimension scale
  - alpha particle observed with the correct energy (but some discrepancy vs simulation in the yield)

- **Radiobiology**
  - Radiobiological effect observed with different beam energies (60 MeV and 150 MeV), different cellular lines and different target molecules (BPA)
  - Bystander effect observed for the first time
  - Also observed at monocromatic energy direct on cells at the correct energy (no contribution from neutron on ^{10}B possible)

Goals of WP2 (imaging)

- Evaluate bio-distributions of borated & fluorinated tracers using ^{19}F-MRI

- ^{19}F-MRI performances limited by low SNR ratio

- Possible **hardware improvements** to ^{19}F-MRI
  - new antenna (low noise)
  - software defined radio technology for signal digitization
  - new pre-amp & cooling

test-stand: 0.35 T scanner

- Choice of fluorinated molecules
  - tests on animals to have samples with correct concentrations

9T spectrometer

- Possible **sofware improvements** to ^{19}F-MRI
  - use of deep learning to denoise and analyse images

^{19}F-MRI vs ^{1}H-MRI

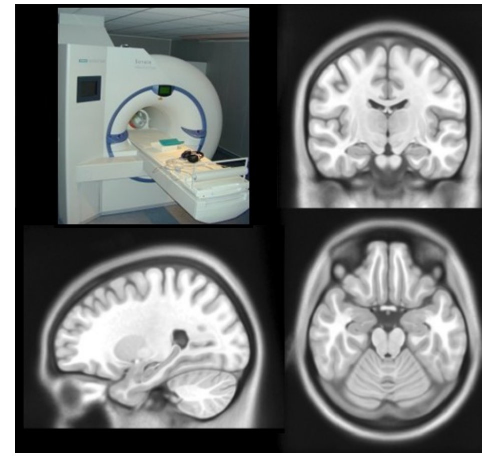
- Conventional clinical MRI = ^{1}H-MRI

  - detection of signals from mobile protons of water or lipids
  - high spatial resolution and excellent soft tissue contrast

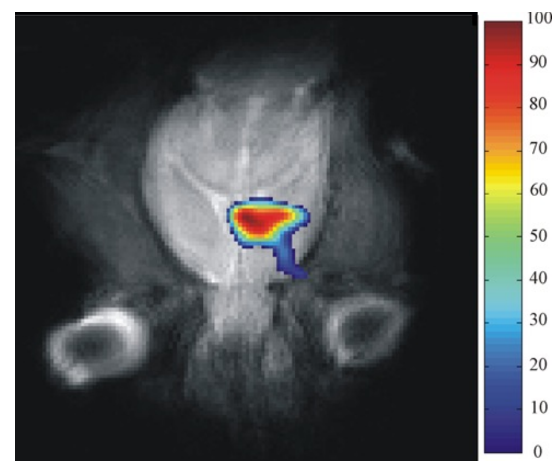
- **^{19}F has extremely favorable magnetic properties:**

  - 100% natural abundance, spin ½
  - gyromagnetic ratio very close to ^{1}H (40.08 vs 42.58 MHz/T)
  - only trace amounts in human body
    => **can specifically detect administered ^{19}F-containing compounds without background signal**

^{1}H-MRI



^{1}H-MRI + ^{19}F-MRI

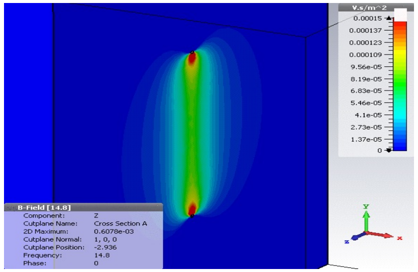


P. Porcari, S. Capuani, E. D'Amore *et al.* 2008 *Phys. Med.* Biol.

Hardware improvements to ^{19}F-MRI

New Antenna

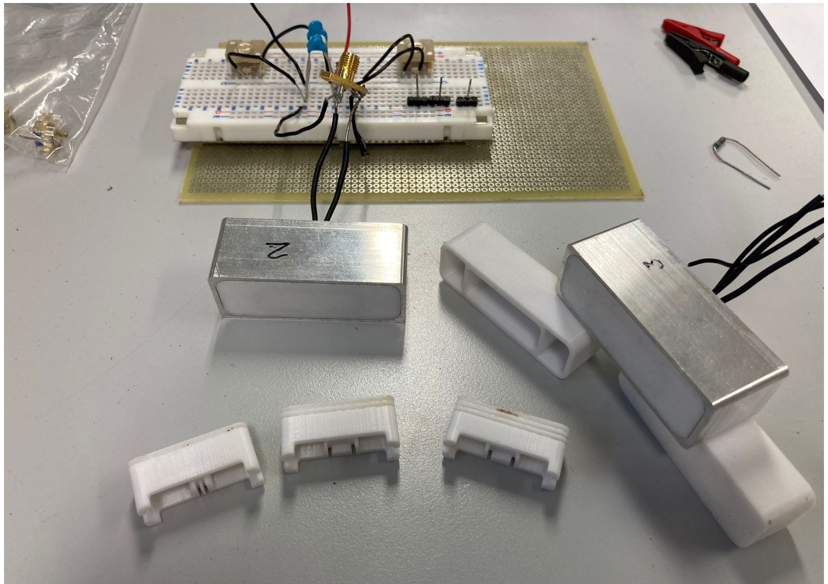
L. Ficcadenti

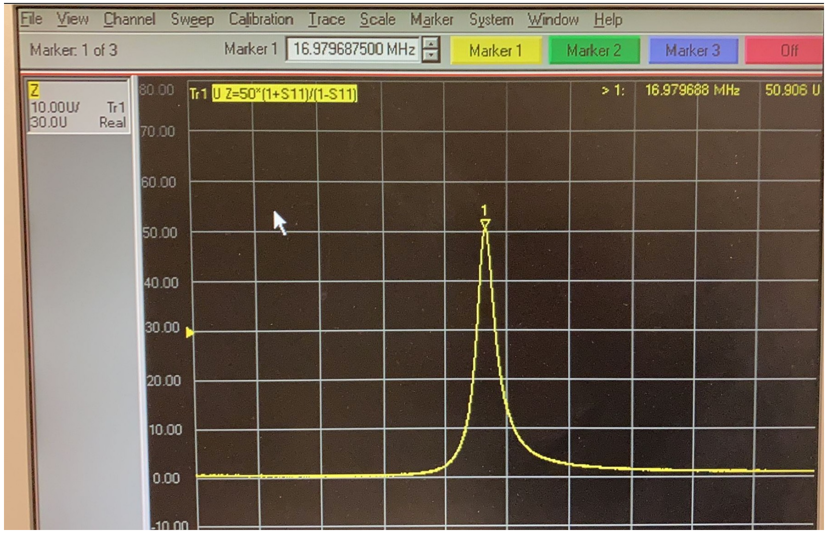


- Goal: better SNR ratio
- Antenna designed with CST simulations
- Housing realised with non magnetic materials

- Prototypes of the new antenna have been realized (1-2-3 loops)
- EM characterization done

- Next tests: First tests on the Brucker 0.35T spectrometer





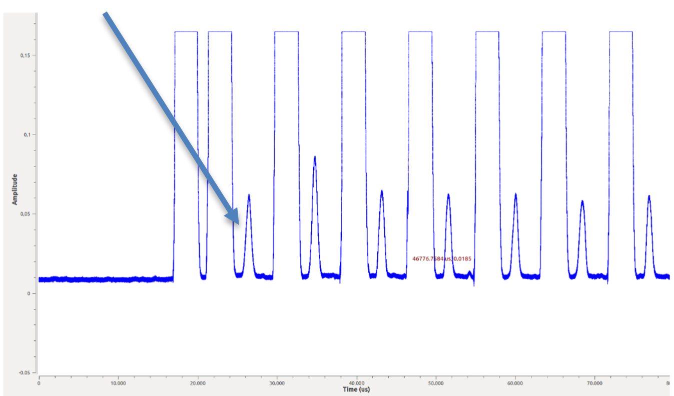
Software Defined Radio (SDR)

- Goal: use SDR to process NMR signal before demodulation (this was not found possible on the low field Brucker scanner)
- SDR system bought and installed
- We finalized the SDR setup with GNU-radio and gr-MRI software

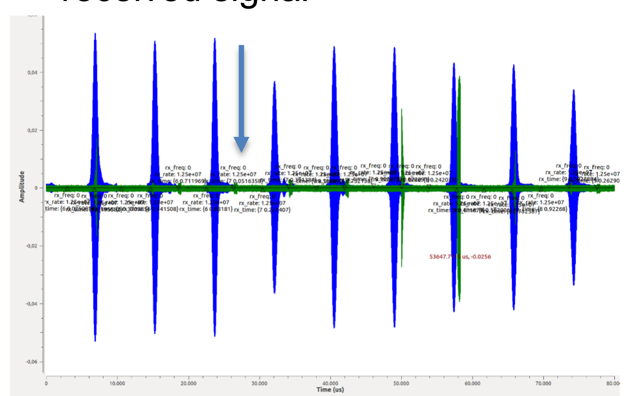
- Some data were taken from the above scanner (echo signals)
- Base signal receiving, transmission and manipulation implemented in GNU radio

- Next: use new antenna to acquire signals before demodulation

echos from low field scanner



received signal



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Choice of fluorinated molecule

Choice of fluorinated molecule

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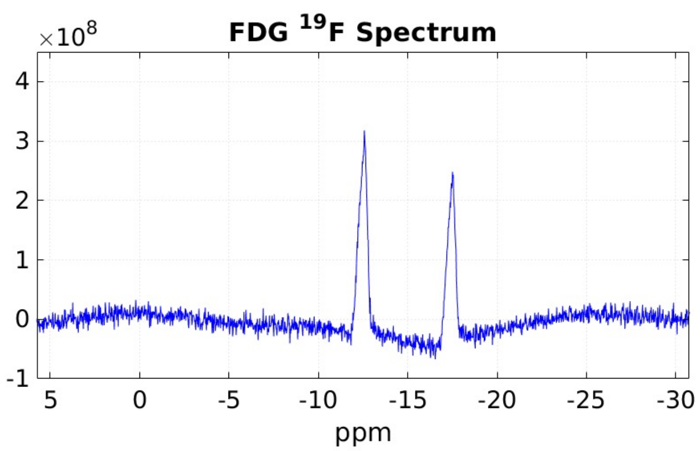
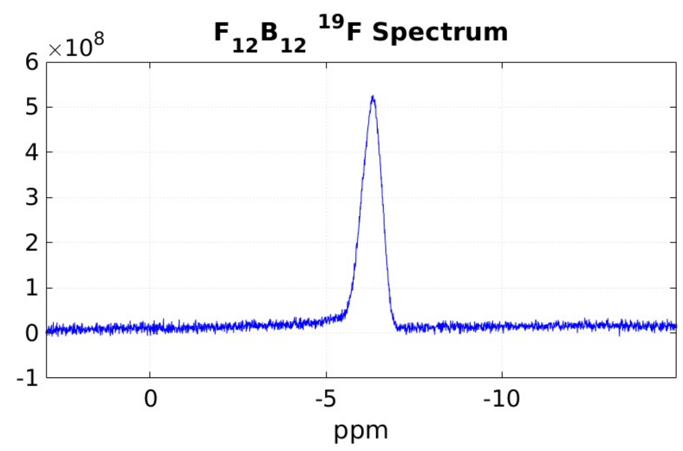
A.Ciardiello
D. Rotili
ISS

- 4 different fluorinated molecules were taken into consideration and studied in MR-Spectroscopy

- FDG <= not ok for imaging (double peak in MRS)
- 5F-phenylanalina <=not ok for imaging (multiple-peaks in MRS)
- **F-BPA** <= **ok for imaging, contains B, internalized by PANC, not cytotoxic: we will use it for animal tests and we propose it for radiobiological tests**
- $F_{12}B_{12}$ <= ok for imaging, not ok for internalization, toxic





Internalization Measurements

- F-BPA internalization fraction in PANC-1 in agreement with 3 different techniques:

  1) neutron autoradiography (Pavia)

  2) liquid chromatrography with mass spectroscopy (Caserta)

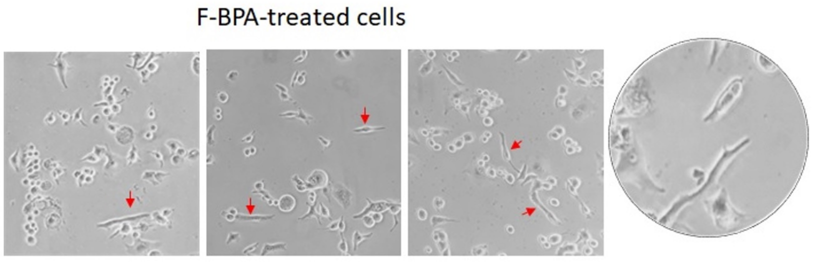
  3) ^{19}F Magnetic Resonance Spectroscopy (Roma)

1)

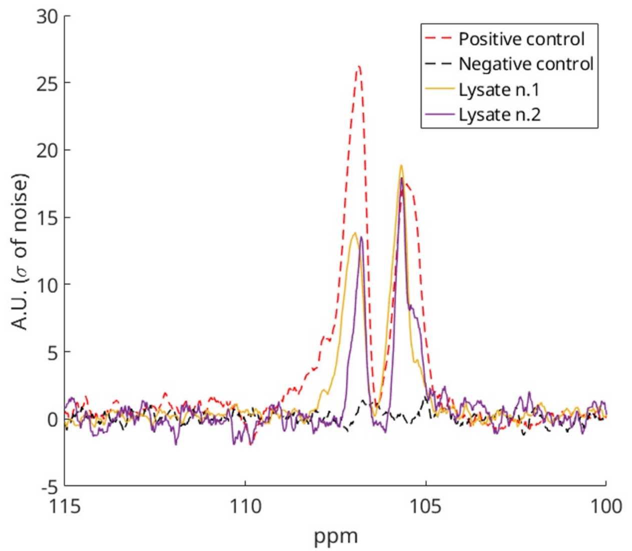
Table 1 ^{11}B concentration obtained by quantitative neutron autoradiography

Experiment	Sample	^{11}B in ppm (mean±SD)	Internalized fraction (mean±SD)
1	1	63 ± 2	0.52 ± 0.03
1	2	66 ± 2	0.55 ± 0.03
1	3	68 ± 2	0.56 ± 0.03
2	1	56 ± 2	0.47 ± 0.04
2	2	54 ± 2	0.45 ± 0.04
2	3	55 ± 2	0.46 ± 0.04

2) f= 0.524±0.008



3) f= 0.5 ± 0.1



Internalization measurements

- Paper with in-vitro internalization measurements in PANC-1 under revision by Physica Medica

Multimodal evaluation of ^{19}F-BPA internalization for BNCT and PBFT potential applications in pancreatic cancer cells

Abstract

Purpose: One of the major obstacles to the application of therapies such as Boron Neutron Capture Therapy (BNCT) and Proton Boron fusion therapy (PBFT) concerns the measurement and monitoring of BPA concentration in cancer cells. The objective of the present study was to evaluate the in-vitro internalization of 2-fluorinated-4-boronophenylalanine (^{19}F-BPA) in the PANC-1 cell line for the potential application of BNCT and/or PBFT in pancreatic cancer. ^{19}F-BPA carrier has the advantage that its bio-distribution may be in principle monitored in vivo using ^{19}F- Magnetic Resonance (MR).
Methods and Materials: The ^{19}F-BPA internalization in PANC-1 cells was evaluated using three independent techniques at ^{11}B concentration equal to 120 ppm: neutron autoradiography, which quantifies boron, liquid chromatography hyphenated to tandem mass spectrometry and UV-DAD which quantifies ^{19}F-BPA molecule, and ^{19}F-MR Spectroscopy, which detects fluorine nuclei.
Results: Our in vitro studies suggested that ^{19}F-BPA is well internalized by PANC-1 cells. The three methods provided consistent results of about 50% internalization fraction at 120 ppm. Small variations (less than 15%) in internalization fraction mean value are mainly dependent on the proliferation state of the cells.
Conclusions: The ability of ^{19}F MR Spectroscopy to study ^{19}F-BPA internalization was validated by well-established independent techniques. The multimodal approach we used suggests ^{19}F-BPA as promising BNCT/PBFT carrier for the treatment of pancreatic cancer.

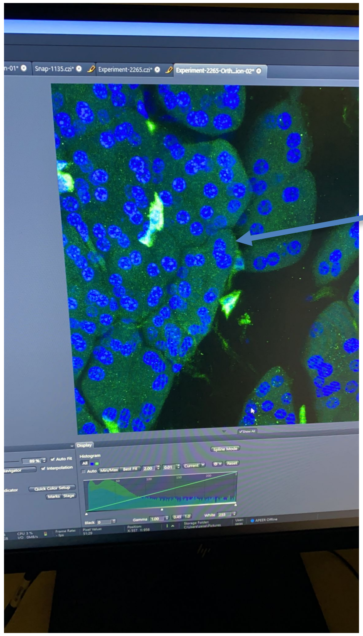
Animal model

L. Milazzo
D. Rotili
F. Vulcano

- 3 NOD scid mice
- PANC-1 cells orthotopically injected in the pancreas:
  - mouse1 and mouse2 (mouseCRT = control)
- After 3 weeks 200μL of F-BPA in fructose solution administered throught tail vein = 200mg/Kg in line with reported treatments in BNCT
- after 45-50 minutes mice sacrificed

Sample in RED have been studied with MRS, others sent to Pavia for neutron-autoradiography

Sample	**Mouse CRT**	**Mouse 1**	**Mouse 2**
Blood (μL)	650	550	530
Liver (g)	1.45	1.06	1.36
Spleen (mg)	52	18	38
Kidney (mg)	393	364	464
Pancreas (mg)	130	88	149
Fat (mg)	162	104	187
Skin (mg)	78	128	186
Lungs (mg)	180	132	185
Heart (mg)	156	143	132
Stomach (mg)	290	248	365
Genitourinary sys. (mg)	219	266	323



PANC-1
cell in mouse4
pancreas at
immunohisotochemistry

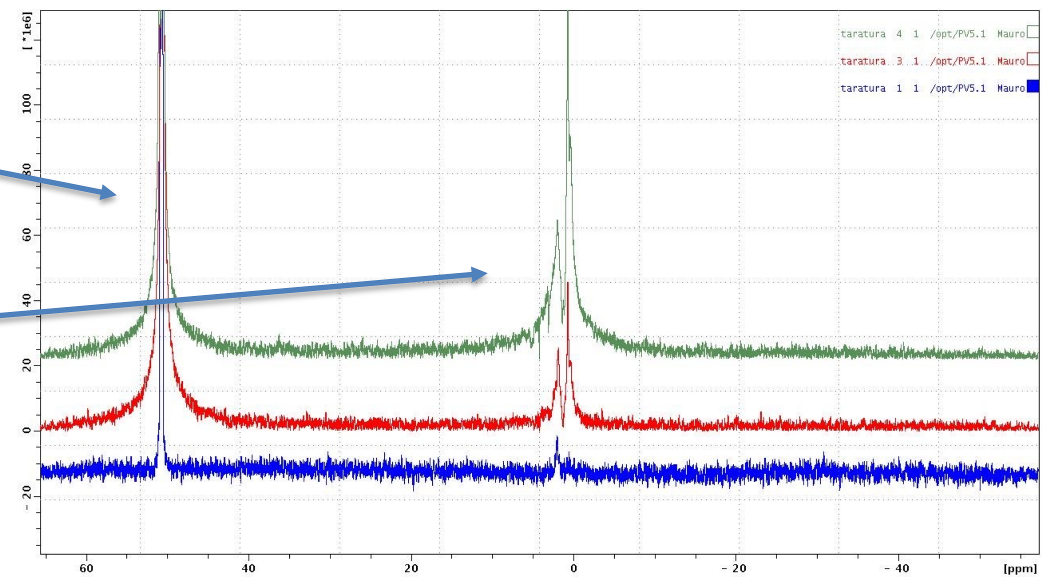
Courtesy of
A. Catizzone

MRS Calibration

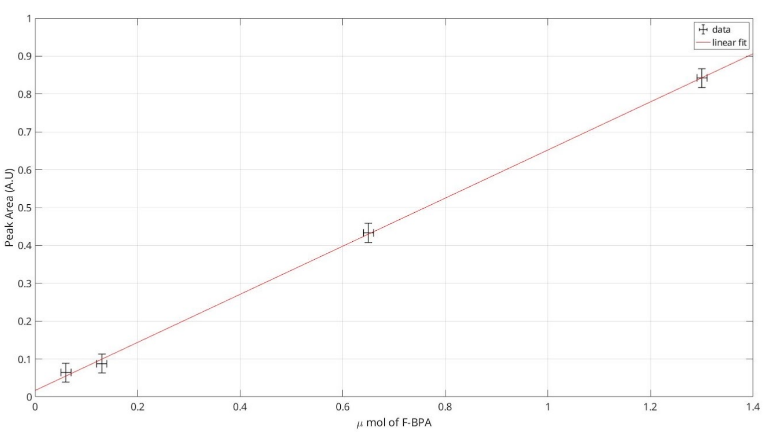
S. Capuani
A. Ciardiello

PFTP-DOPA: external standard

F-BPA at different concentrations



Calibration curve

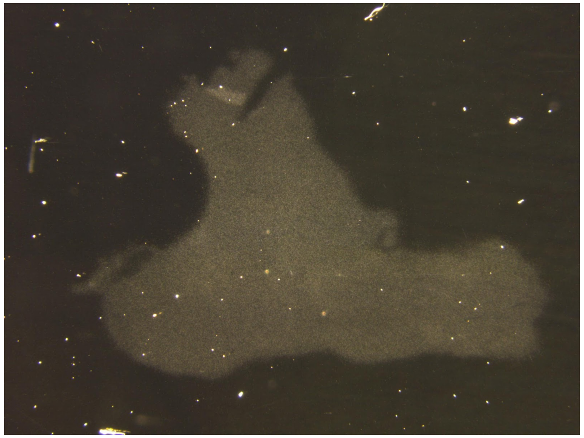


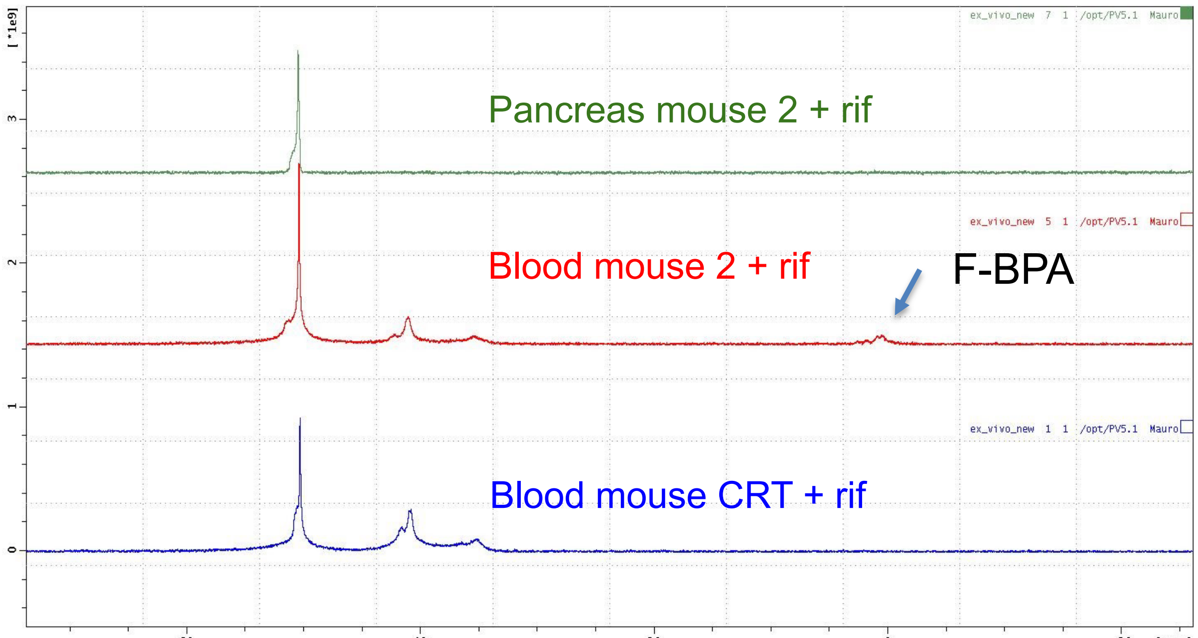
	uMol FBPA	Area ratio	SNR rif	SNR F-BPA
S1	1.30	0.84±0.03	115.2	47.2
S2	0.65	0.43±0.03	123.1	17.2
S3	0.13	0.09±0.03	138.4	4.0
S4	0.06	0.05±0.03	109.3	3.4

Results

S. Capuani
A. Ciardiello

- The only samples where we find F-BPA signals are blood in mouse2, genitourinary mouse2, Kidney mouseCRT (at the limit of our sensitivity)
- Neutron autoradiography in pancreas mouse1 finds very little Boron ($1ppm\ ^{10}B$ => $5ppm\ ^{11}B$)
- Checks underway (spectrometer turned off in March=> now back in operation





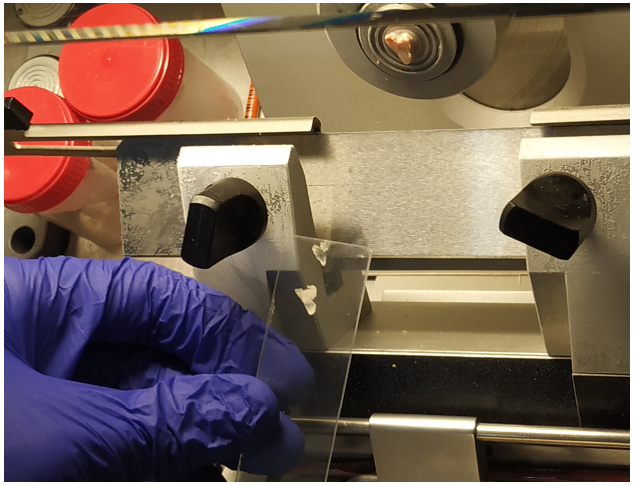
I. Postuma
S . Bortolussi
& Pavia group

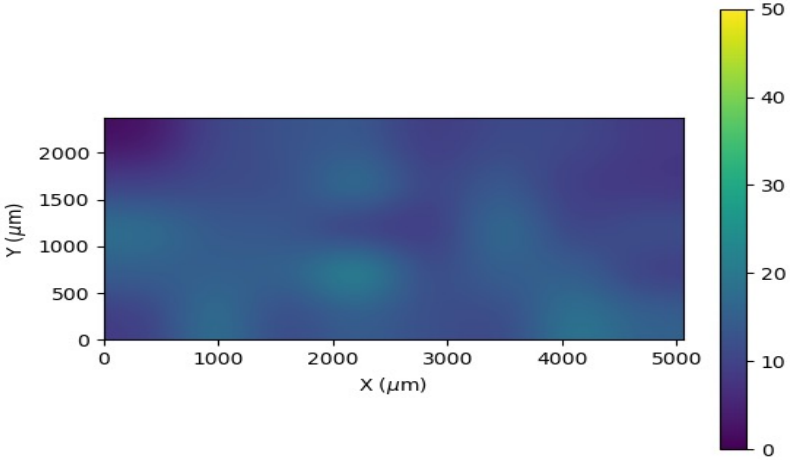
Results: mouse4

I. Postuma
S . Bortolussi
& Pavia group

- We analyzed another mouse doubling the second dose => 400mg/Kg
- We observe ~50ppm of ^{11}B

Organ	^{10}B ppm	Err ppm	^{11}B ppm	Err ppm
Pancreas 1	13.8	0.5	**55**	2
Pancreas 2	11.7	0.5	**47**	2
Pancreas 3	12.3	0.5	**49**	2
Pancreas 4	12.7	0.5	**51**	2
Fegato 1	9.6	0.5	38	2
Fegato 2	10.5	0.5	42	2





Future Measurements

- High-field spectrometers back to operation in Fermi building
- Understand reason of low uptake in mouse pancreas
- We want to perform new in-vitro and ex-vivo measurement with improvements in the protocol for a better quantification:

- reduce resonance line broadening
  => apply better shimming and field locking to the spectromenter to reduce field disomogeneities
  => improve sample preparation i.e."extract" to reduce impact of polar macro-molecules (proteins)
- use an internal standard (reference molecule mixed with sample)
- PFTB-DOPA:
  => see if enhances F-BPA uptake in PANC-1

perfluoro-tert-butoxy 3,4-dihydroxy-L-phenylalanine

PFTP-DOPA

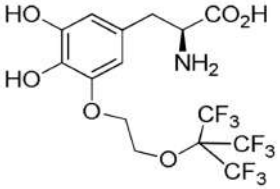


Image analysis: deep learning based denoiser

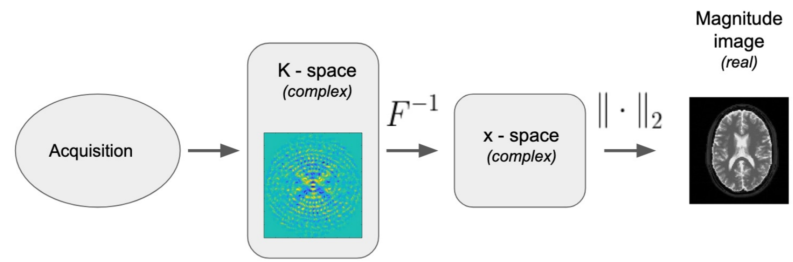
Denoiser in k space

A. Ciardiello
S.Giagu

- Noise in images is Rician distributed, not Gaussian

- We developed a denoiser in k- space
  - noise is gaussian
  - drawback: not always easily acces

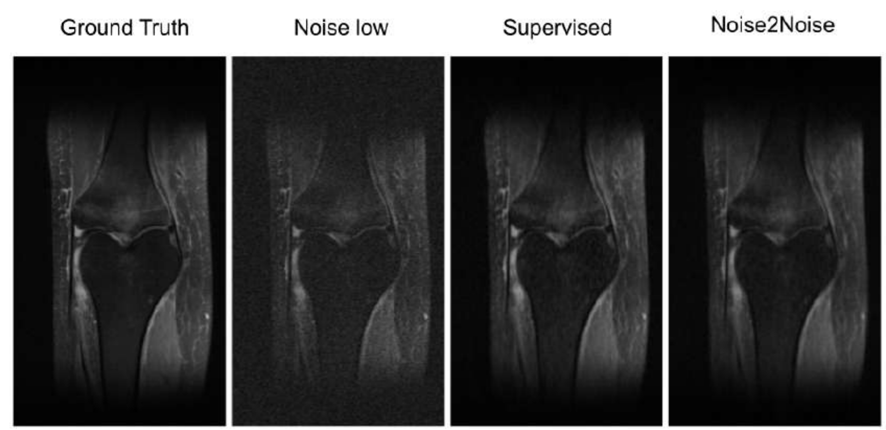
- Residual learning scheme
  - Learn noise not signal:
  - Less prone create artifacts



- Test on public "Fast-MRI" datasets, 40000 images
  - ^{1}H-MRI multicoil dataset with access to k-space
  - high resolution images (ground truth)+ add noise

- We find improvements applying the denoiser to these images (better PNSR)

- Need to check on ^{19}F-MRI images



Summary and Perspectives

- Developed a new antenna and an SDR-based system to improve SNR ratio in ^{19}F-imaging

  - will perform tests on low field scanner to check actual performances

- Delected F-BPA as F-B tracer and studied its internalization in PANC-1 (case study) => 50% internalization

- Performed first tests for quantification F-BPA in ex-vivo mice models at therapeutic doses (MRS)
  - will repeat in-vitro and ex-vivo tests with improved quantification technique
  - study PFTP-DOPA as F-BPA uptake enhancer

- Develop deep learning based denoiser in k-space for MRI images
  - tested on low SNR ^{1}H-images
  - need to test on real ^{19}F images => will try to accumulate a sufficient number of images