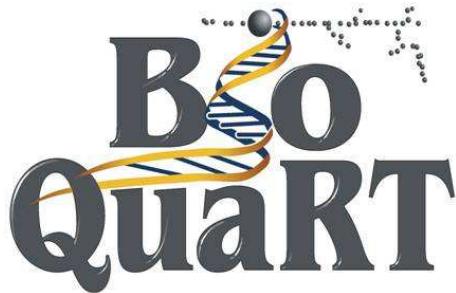# BioQuaRT

Quantification of the production yield of reactive oxygen species and their 2D and 3D distribution in ion tracks

Peter Sharpe, Caterina Minelli, David Crossley, Giuseppe Schettino, Hugo Palmans

WP3 Indirect Effects

"… there would appear to be rather strong grounds for believing that some forms of biological damage resulting from exposure to X radiation are … due to radicals. … Would that I knew which they were"

L H Gray, 1953

Gray LH. The initiation and development of cellular damage by ionizing radiations. The thirty-second Silvanus Thompson Memorial Lecture. Br J Radiol 1953;26:609–18.

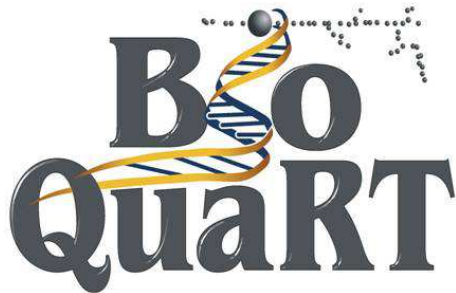Source: P Wardman - 2008 Silvanus Thompson Memorial Lecture

WP3 Indirect Effects
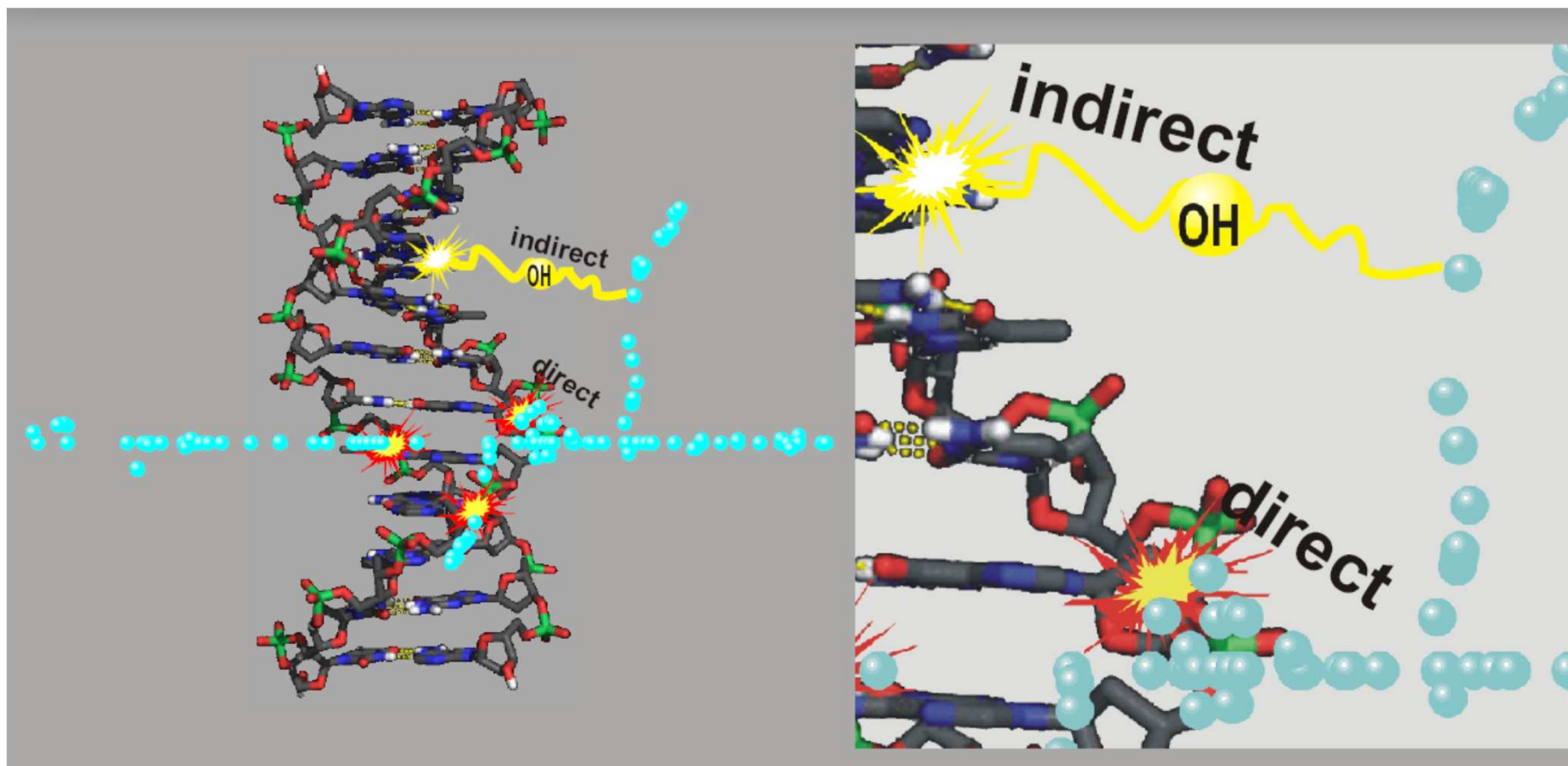
WP3 Indirect Effects

Task 3.1: Quantifying reactive oxygen species in bulk solution

Task 3.2: Spatial distribution of reactive oxygen species

## Task 3.1: Quantifying reactive oxygen species in bulk solution

The aim of this task is to develop methods to quantify radiation induced biologically significant reactive species, particularly reactive oxygen species (ROS), by the use of specific probes and techniques.

An essential aspect is a critical literature review of the information gained over many decades and will concentrate on the identification of successful techniques and the consistency of data relating radiation chemical yields to radiation type and energy.

Work Package 3 – Indirect Effects

Quantifying biologically significant reactive species in bulk solution

- Critical literature review.

- Correlate species yields with LET and biological effectiveness.

- Identify selective probes for biologically significant species, particularly reactive oxygen species.
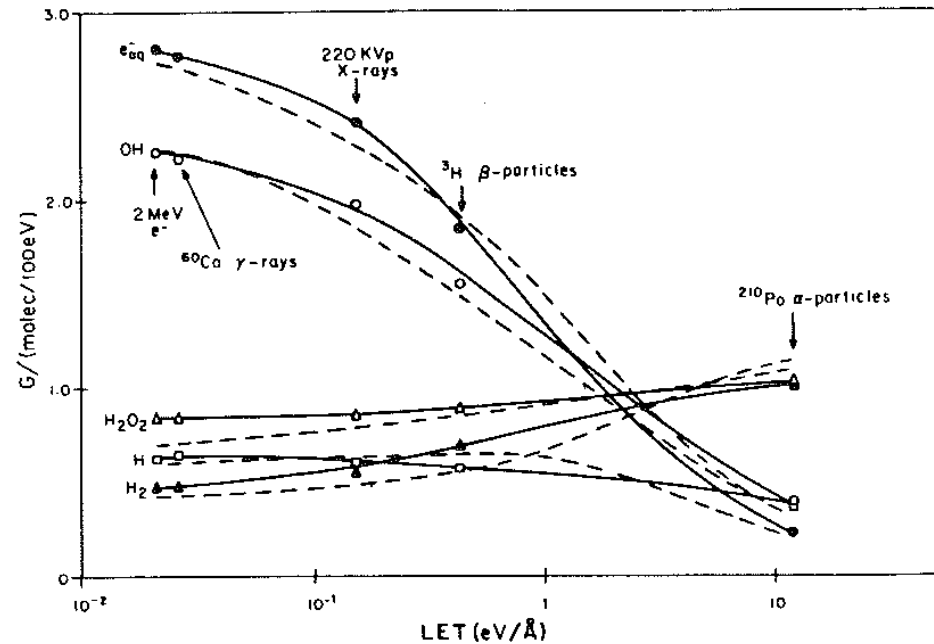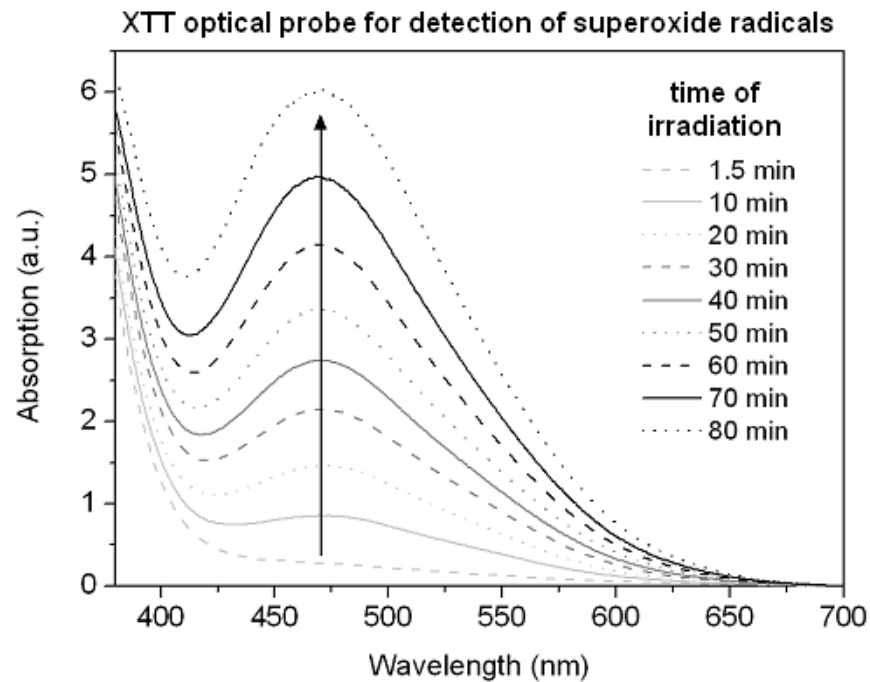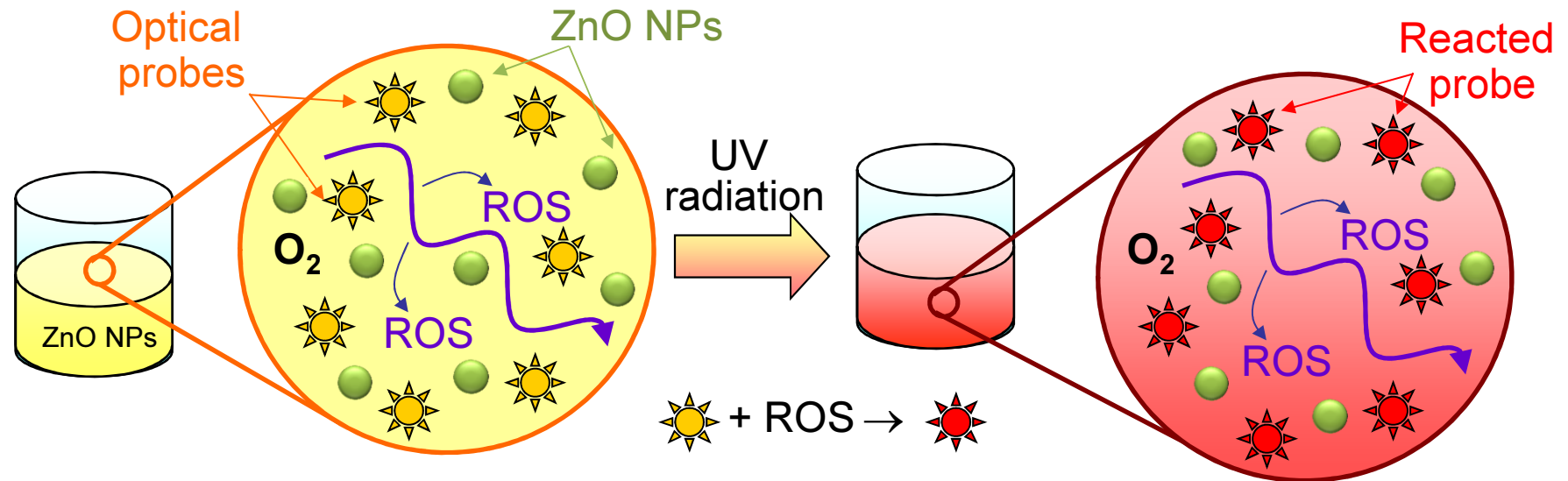
Preliminary probes for ROS detection

Probe	Reacted probe	ROS detected	Absorption	Fluorescence
KI	Tri-iodide	unspecific	352 nm	no
XTT*	XTT formazan	$\cdot O_2^-$	470 nm	no
AmplexRed**	Resorufin	H_2O_2	560 nm	590 nm
Coumarin	Hydroxyl-coumarin	$\cdot OH$	277 nm	460 nm

* 2,3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt

** Amplex Red reacts with H_2O_2 in a 1:1 stoichiometry in presence of horseradish peroxidase to produce red-fluorescent resorufin

Example: detection of photo-generated probes

Optical probes

ZnO NPs

Reacted probe

UV radiation

O₂   ROS   ROS

☀ + ROS → ✦

Increasing O₂⁻ radical concentration

XTT optical probe for detection of superoxide radicals

time of irradiation
- 1.5 min
- 10 min
- 20 min
- 30 min
- 40 min
- 50 min
- 60 min
- 70 min
- 80 min

Absorption (a.u.)

Wavelength (nm)

Molecular probes with tailored optical properties used to quantify NP-enhanced photo-production of superoxide radicals and other ROS

C Minelli et al, Nanomaterials and the Environment 2012, 40-47 (2013)

WP3 Indirect Effects

Species specific probes

Coumarin selected for initial study:

- Selective reaction with OH·

- Stable fluorescent product (higher sensitivity *cf*. optical absorption).

- Radiation chemistry well understood.
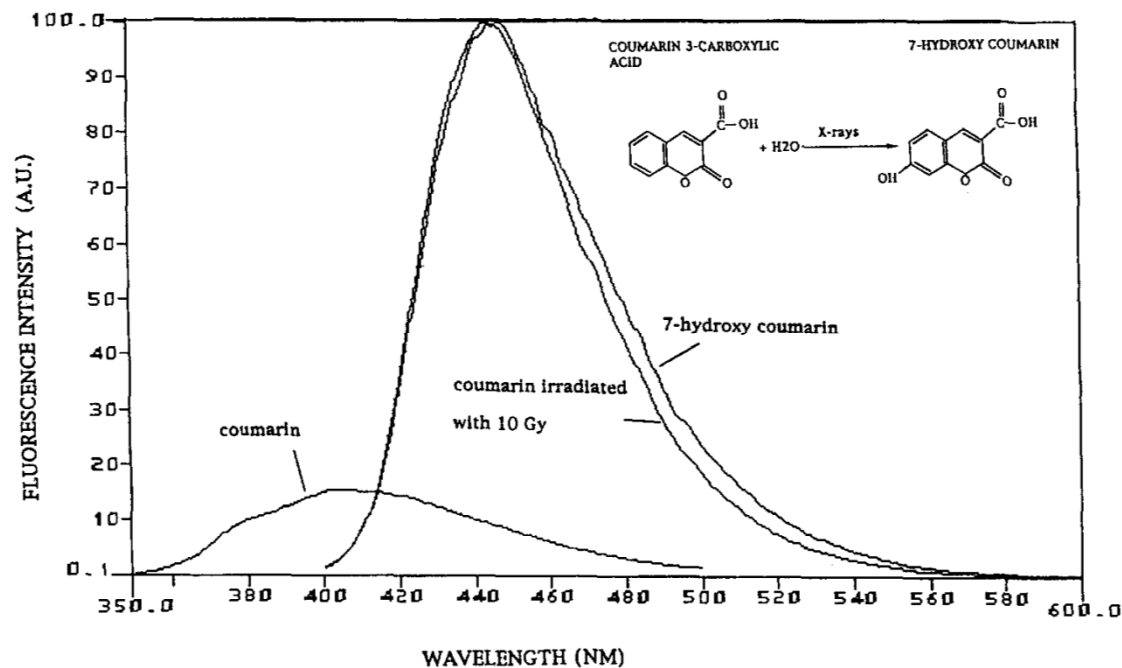
WP3 Indirect Effects

Task 3.2: Spatial distribution of reactive oxygen species

The aim of this task is to investigate options for characterising the spatial distribution of biologically significant reactive species, particularly ROS, in aqueous environments.

WP3 Indirect Effects

**Task 3.2: Spatial distribution of reactive oxygen species**

This may be achieved by:

- direct imaging methods

- electron paramagnetic resonance (EPR) and NMR measurements, that characterise the environment surrounding radiation induced species.

WP3 Indirect Effects

Direct imaging

- Fluorescent probes held in rigid matrix.

- Either organic gels or polymer matrix.

- Image using super-resolution fluorescence microscopy (dSTORM, *direct* stochastic optical reconstruction microscopy)
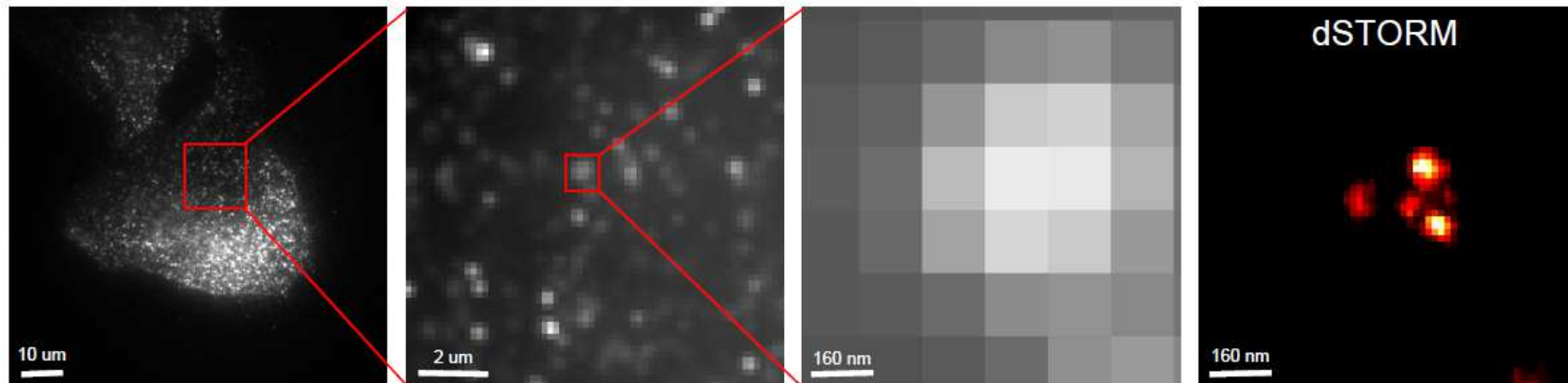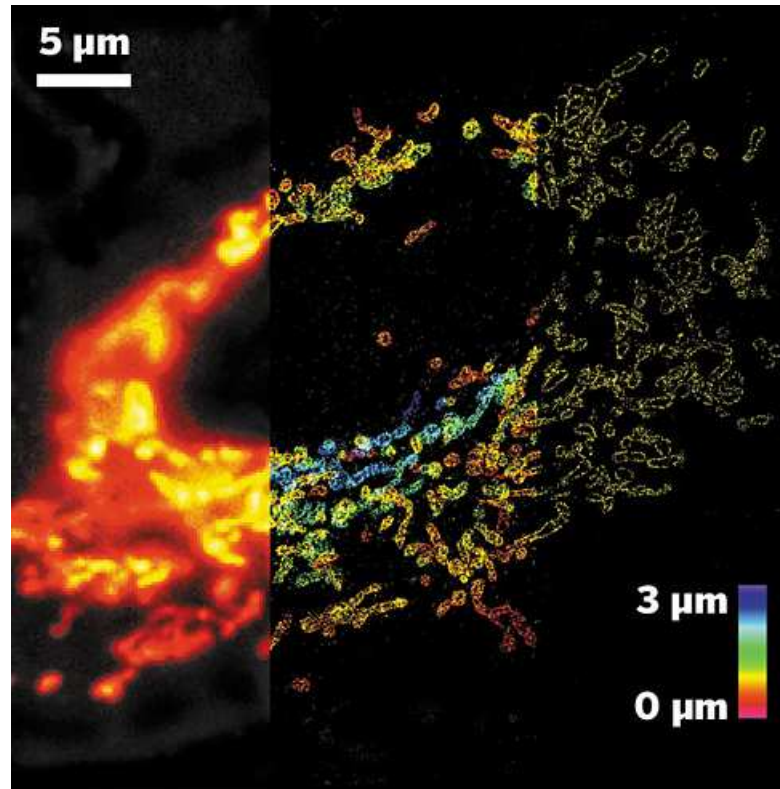

A HeLa cell with fluorescently labeled vesicles (transferrin-Alexa 647) is shown. These are densely-packed an approximately 50-100 nm in size. The dSTORM image shows the same region and illustrates the improvement in resolving power.

http://www.npl.co.uk/publications/science-posters/storm-ing-through-the-diffraction-limit

http://www.npl.co.uk/publications/science-posters/application-of-super-resolution-imaging-to-the-endocytic-pathway

by Alex Knight, Daniel Metcalf and coworkers

3-D dSTORM resolution

by Xiaowei Zhuang

3-D STORM provides details of the mitochondrial network in a mammalian cell. The image shows conventional fluorescence (left), 3-D STORM color-coded by depth (center), and an x-y cross section of 3-D STORM (https://pubs.acs.org/cen/science/87/8736sci4.html)

WP3 Indirect Effects

EPR / NMR methods

- Fine structure of EPR / NMR spectra is influenced by the local environment around the molecule.

- Local environment around radiation induced species will be dependent on the density of energy deposition and subsequent chemical reactions.

- EPR / NMR spectral characteristics may provide information on LET as well as dose.

WP3 Indirect Effects

Alanine radical spectra following Co–60 and Xe ion beam irradiation
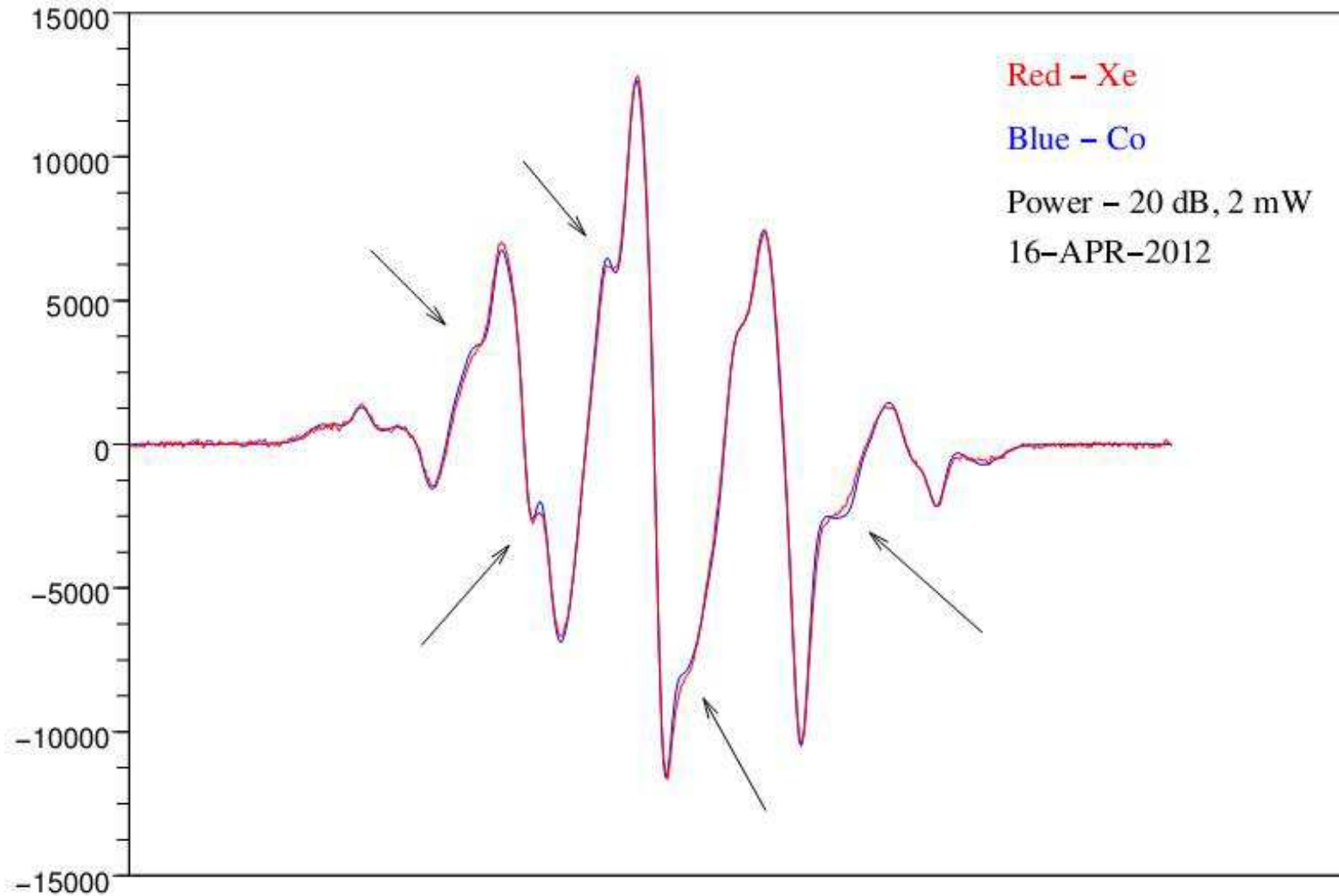
BioQuaRT

Work Package 3

Thank you