ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2023005

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Co-investigator (*)	Professor Marco Laurati, CSGI, ITALY
Co-investigator	Dr Mariana Vide Tavares, University of Coimbra, PORTUGAL
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Experiment title	ELECTRON MICROSCOPY FOR DISCRIMINATING HEALTHY FROM CERVIX CANCER HUMAN TISSUES
MRF Instrument	**High Resolution TEM**
Access Route	Direct Access
Science Areas	Biology and Bio-materials, Chemistry, Medicine
Sponsored Grant	None
Grant Title	-
Start Date	-
Similar Submission?	-
Industrial Links	-

Days requested: 2
Previous GP Number: No
DOI: -
Sponsor: -
Grant Number: -
Finish Date: -

Non-Technical Abstract

Normal-to-cancer (NTC) transformation, still an ill-understood process, is associated to variations in the cellular biochemistry which prompt morphological changes (detected by histological analysis). Furthermore, healthy-to-cancer transition is related to the biomechanical properties of cells within tissues, strongly dependent on the dynamics of interfacial water and known to play a fundamental role in normal cellular activity. Building on the success of previous studies by vibrational microspectroscopy (FTIR/Raman) and neutron scattering techniques in human cancer and non-cancer cells/tissues, this proposal aims to apply SEM and TEM for probing healthy and cancer human tissues, This is an innovative approach for identifying particular features associated to malignancy, coupling biochemical, biophysical and morphological data.

Publications

R. Senesi et al., Antioxidants 10 (2021) 643. DOI: 10.3390/antiox10050643

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IM@IT E-platform: No

Instruments	**Days Requested:**
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Start Date	**Finish Date:**
Similar Submission?	
Industrial Links	

Sample record sheet

Principal contact Professor Marco Laurati, CSGI, ITALY
MRF Instrument **High Resolution TEM** **Days Requested:** 2
Special requirements:

SAMPLE

Material	human tissues from cervix cancer - 300 nanometer slices, frozen	human tissues from cervix (healthy) - 300 nanometer slices, frozen	-
Formula	NA	NA	-
Forms	Solid	Solid	
Volume	10 cc	10 cc	
Weight	500 mg	500 mg	
Container or substrate	sample holder for TEM measurements - Cryo-EM grid	sample holder for TEM measurements - Cryo-EM grid	-
Storage Requirements	freezer at -80 ºC	freezer at -80 ºC	-

SAMPLE ENVIROMENT

Temperature Range	190 - 195 K	1000 - 1000 K	-
Pressure Range	1000 - 1000 mbar	190 - 195 mbar	-
Magnetic field range	NA - NA T	NA - NA T	-
Standard equipment	-	-	-
Special equipment	-	NA	-

SAFETY

Prep lab needed	Yes	Yes	-
Sample Prep Hazards	No	No	-
Special equip. reqs	No	No	-
Sensitivity to air	No	No	-
Sensitivity to vapour	No	No	-
Experiment Hazards	No	No	-
Equipment Hazards	-	-	-
Biological hazards	NO	No	-
Radioactive Hazards	NO	No	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Removed By User	Removed By User	-

ELECTRON MICROSCOPY FOR DISCRIMINATING HEALTHY FROM CERVIX CANCER HUMAN TISSUES

Background and Context:

Cancer is a worldwide health problem, being the second leading cause of death globally (9.6 million deaths in 2018) and expected to rise up to 22 million cases *per* year within the next two decades [1]. An effective chemotherapy is therefore a pressing medical and social need, aiming at targeting neoplastic cells with minimal damage on healthy tissue. However, a successful development of novel anticancer agents relies on a thorough understanding of the carcinogenesis process, *i.e.* of the specific biochemical and biophysical mechanisms responsible for the normal-to-cancer (NTC) transition, that may enable both an early diagnosis and the development of selective and improved drugs. Recently, attention has turned to the biophysics of the cancer state, shedding a new light on cancer beyond the recognised biochemical and genetic variations associated to malignancy, with a view to unravel the transition from healthy to cancer as well as from localised tumours to metastatic states. At present, cancer diagnosis and staging (according to the American Joint Committee on Cancer) are based on the morphological alterations of the tissue, tumour size, grade and location [2]. Although this current practice is functional, the overall process is still time-consuming and subjective, which carries risks for the patients.

Vibrational spectroscopy has arisen as a promising tool to assist histological diagnostic methods, allowing cancer detection at an early stage in a fast and non-invasive way [3-5]. FTIR and Raman microspectroscopy (including synchrotron-radiation FTIR and AFM-FTIR) have been applied by the applicants to identify spectral biomarkers of disease in human cancer cells and tissues [6-8], at the "Molecular Physical-Chemistry" R&D Unit of the University of Coimbra (Portugal, http://www.ci.uc.pt/qfm/), the Diamond Light Source and the ISIS Neutron and Muon Source (UK, https://www.diamond.ac.uk/ and https://www.isis.stfc.ac.uk/). Instead of monitoring morphological differences as in current histopathological methods, these methods probe chemical variations, which usually arise earlier, with high sensitivity and specificity and unmatched spatial resolution. In addition, neutron-based techniques (inelastic and quasi-elastic neutron scattering, INS and QENS) were used to probe changes in the dynamical activity of intracellular water between healthy and cancerous human, having unveiled a higher plasticity for malignant states, mainly regarding the cytoplasmic water dynamics [9-14] (Fig. 1).

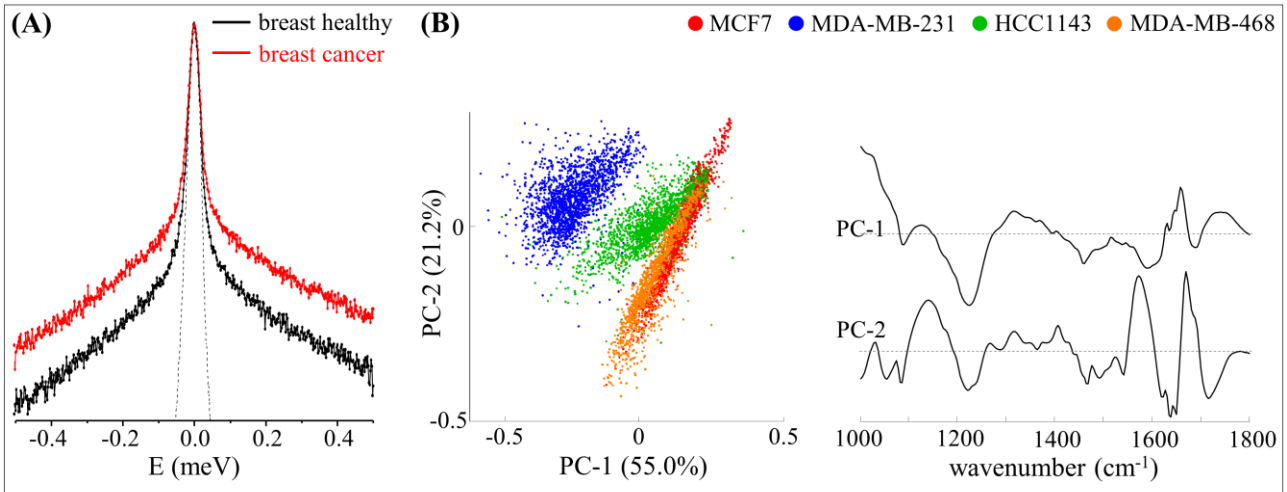

Figure 1 – (A) Normalised QENS profiles (at 310 K) for human breast cancer and healthy tissues [14].
(B) Principal component analysis (PCA) of FTIR data for distinct human breast cancer cell lines [7].

Since specific changes are known to occur in tissues upon NTC transition (*e.g.* in connective tissue stroma) [15], analysis of the tissue framework, with high sensitivity and spatial resolution, is prone to deliver crucial data on the processes underlying malignancy. Electron microscopy techniques, both scanning (SEM) and transmission (TEM), are extremely suitable for the study of these types of heterogeneous biological systems, allowing imaging at a higher resolution than when using light microscopy and therefore yielding very detailed information on morphological changes [16-18]. While the former provides data on surfaces and their properties, the latter yields information on the tissue interior layers. Moreover, high resolution TEM instruments (such as the Glacios Cryo-TEM microscope available at Florence University) enable 3D tomography. The combined SEM and TEM experiments envisaged in the present study are expected to

provide an accurate understanding of the tri-dimensional structure and organisation of the tissue components in both non-malignant and tumour specimens, hopefully leading to the identification of biological markers that may discriminate diseased from healthy states.

Building on the success of the previous FTIR/Raman and QENS/INS experiments carried out by the authors to discriminate malignant from non-malignant specimens, the present study aims to go one step further and apply high-resolution SEM and TEM to normal and diseased (tumourigenic) human tissues. Hence, the data on biochemical/metabolic changes, as well as dynamical variations, occurring during carcinogenesis will be coupled to specific morphological features. The main goal of this approach is to attain an improved understanding of the biochemical and biophysical differences between diseased (cancer) and healthy tissue, which may help to better apprehend the mechanisms of neoplastic transformation, cancer invasiveness and metastasis. In addition, these results (at the nanometer lengthscale) may assist the interpretation of images delivered by MRI, which is currently one of the most widely used tumour diagnosis technique based on differential water diffusion properties (at the μm and μs scales).

This experiment is within a Masters project of the student Maria Félix, hosted at the "Molecular Physical-Chemistry" R&D Unit of the University of Coimbra, under the subject "Identification of Spectral Biomarkers of Cervix Cancer by Raman and FTIR Microspectroscopy". The samples will be made available from human biopsies, within a protocol already established with the Portuguese Oncology Institute of Porto after approval of this research activity from the respective scientific and ethical committees.

Proposed Experiment:

TEM measurements are envisaged for human tissues from cervix, to assess morphology differences between healthy and cancer specimens. These are anonymised human samples surgically resected at the Portuguese Oncology Institute at Porto, obtained with the informed consent of the patients within the EU ethical and legal guidelines for research in human tissues. In total, tissues from 34 caucasian women were analysed by FTIR and Raman: 24 healthy (aged 44 to 79) and 10 cervical squamous cell carcinoma (aged 38 to 68). In the present work at least two samples from each type (healthy and malignant) will be probed.

The tissues are frozen immediately after resection, cut into 300 nm slices using a cryotome and placed onto a Cryo-EM grid. The tissues will be preserved at low temperature (-80 ºC) prior to analysis. No parafinisation or formalin-fixed samples are foreseen, as formalin treatment has been found to affect the biochemical profile of the tissue (*e.g.* proteins) as well as the intracellular water dynamics [19].

The measurements will be performed at cryogenic temperature. The data will be analysed by the IMOD software (alignment of tomographic images) and the Tomviz software (3D reconstruction and rendering).

2 days are requested for TEM experiments in the Glacios Cryo-EM instrument, at the laboratory for high resolution SEM and TEM of the Chemistry Department of Florence University (Italy), within the ISIS@Mach ITALIA project. The following samples will be measured:

(i) human cervix cancer tissues – 2 samples
(ii) non-tumourigenic human cervix tissues – 2 samples

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