Multidisciplinary Workshop at LSM

18-19 October, 2023

Underground radiobiology at LNGS laboratory

Patrizia MORCIANO

INFN-Laboratori Nazionali del Gran Sasso

on behalf of the Cosmic Silence collaboration

COSMIC SILENCE

Deep Underground Laboratories (DULs) are Interesting facilities to carry out studies at dose rates significantly lower than on the Earth's surface

Compared to that at the Earth's surface, inside DULs, the dose/dose rate contribution due to photons and directly ionizing low-LET (mostly muons) **cosmic rays** can be considered negligible, being reduced by a factor between 10^4 and 10^7 depending upon shielding.

Radiation exposure due to **neutrons** is also extremely low, being reduced by a factor between 10^2 and 10^4 (10^3 at LNGS)

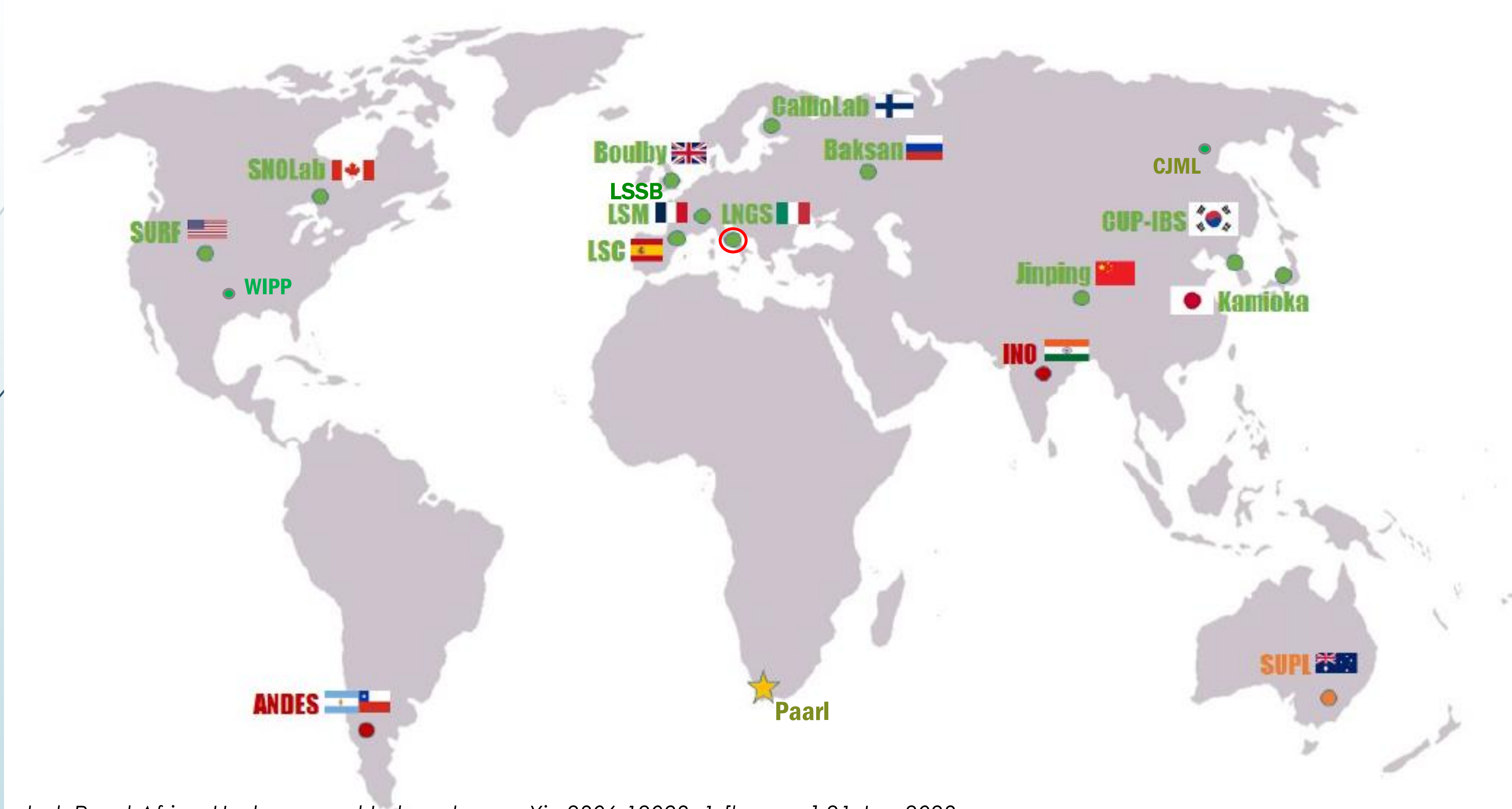
One further contribution to the overall dose/dose rate can come from **radon decay products**, but it depends upon the radon concentration, which can be kept at the same levels of the reference radiation environment by a suitable ventilation system or it can be strongly reduced by radon abatement systems.

Terrestrial gamma rays represent the major contribution to the dose/dose rate inside the DULs.

Lowe et al. Radiat Environ Biophys 2022

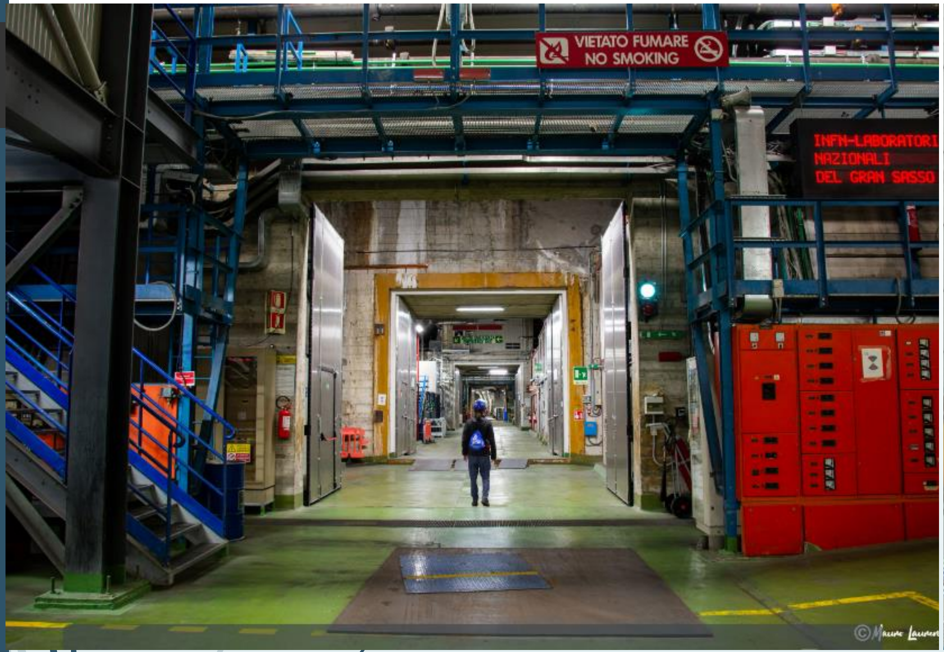
Map of the existing or planned underground laboratories

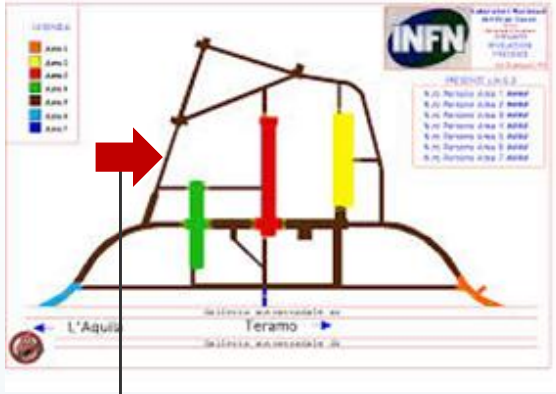
green dots: the operating facilities

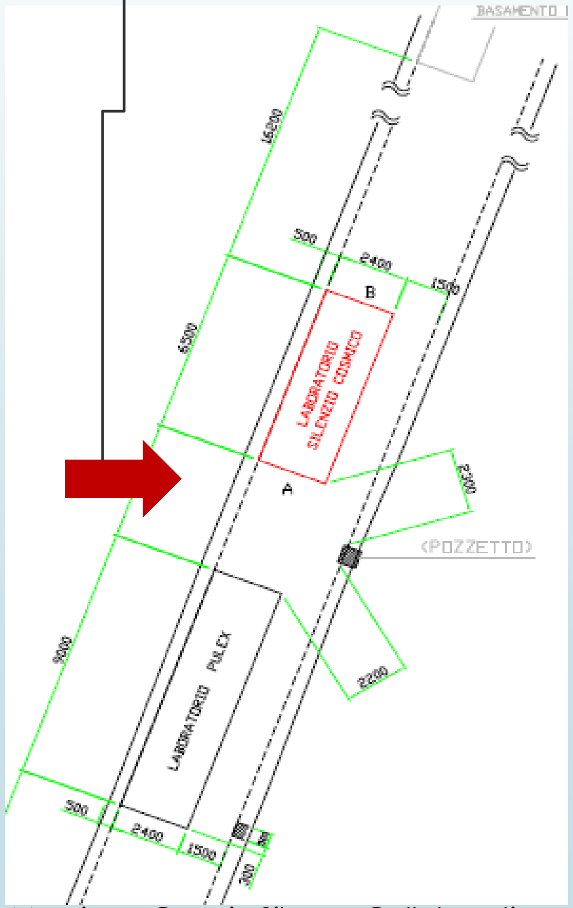


Modified from Adam et al. Paarl Africa Underground Laboratory_arXiv:2306.12083v1 [hep-ex] 21 Jun 2023

The LNGS Underground Laboratory







P. Morciano-Cosmic Silence Collaboration

LNGS represents a unique opportunity for investigating the response of biological systems to below natural radiation background

(extremely low radiation dose/dose rate)

Relevant scenario for both basic and applied science

- All living organisms have to deal with the **natural level of radioactivity on the Earth** as well as with **cosmic rays**. Which is the role of natural variations of background radiation during the Life evolution? How does it contribute to the development of cellular defense mechanisms?

- Underground laboratories give the **opportunity to test the linear no-threshold (LNT) model** currently used in radiation protection, for which stochastic risk is directly proportional to dose and no detriment is expected below the average natural environmental background

Summary of *in vitro* and *in vivo* experiments at LNGS

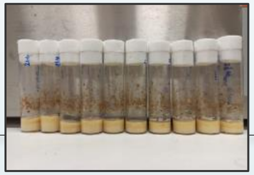
LRE: *Low Radiation Environment*
RRE: *Reference Radiation Environment*

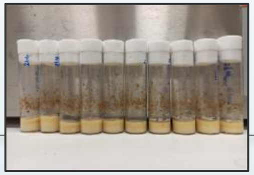
Yeast	**Saccharomyces cerevisiae** *cultured for 1 week (~120 generations) at LRE and RRE (University of Rome)*	**Mutation induction** (*hprt* locus)	**Satta et al., Mutat Res 1995**
Cultured mammalian cells	***Long term experiments (months)***		
	Chinese hamster V79 cells *cultured for up to 9-10 months (>120 generations) at LRE and RRE (RRE: Istituto Superiore di Sanità, Rome; external LNGS laboratory)*	**Cell growth** **Antioxidant enzymes activity** **Apoptosis** **Mutation induction** (*hprt* locus)	**Satta et al., Radiat Environ Biophys 2002** **Fratini et al., Radiat Environ Biophys 2015**
	TK6 human lymphoblasts *cultured for up to 6 months at LRE and RRE (Istituto Superiore di Sanità Rome)*	**Cell growth** **Micronuclei induction** **Antioxidant enzymes activity**	**Carbone et al. Radiat Environ Biophys 2009**
	Short term experiments (weeks)		
	A11 mouse hybridoma cells *(short term experiments, few weeks) RRE (Istituto Superiore di Sanità Rome)*	**Cell proliferation** **caspase-3 activation** **PARP1 cleavage**	**Fischietti et al., Front Public Health 2021**
Fly	***Drosophila melanogaster*** *(RRE: L'Aquila University)*	**Life span** **Ferility** **DNA repair *(mutants)***	**Morciano et al., J. Cell Physiol. 2018** **Morciano et al., Radiat. Res. 2018** **Esposito et al., Front Public Health 2020**
	Drosophila melanogaster (*RRE: external LNGS laboratory*)	**Chromosome breaks** **DNA repair (*mutants*)**	**Porrazzo et al., Int. J. Mol. Sci. 2022** **Morciano et al., Frontiers in Physics 2023**

P. Morciano-Cosmic Silence Collaboration

Experimental approach:

To mantain and compare parallel twin coltures in the two environments



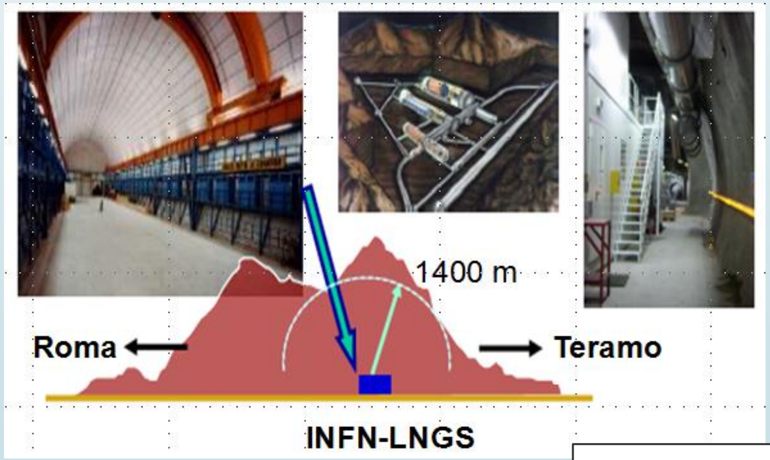


Above ground

INFN-LNGS

Reference Radiation Environment
(RRE)

Underground



Low Radiation Environment
(LRE)

LNGS BIOLOGY FACILITIES

Above ground | **Underground**

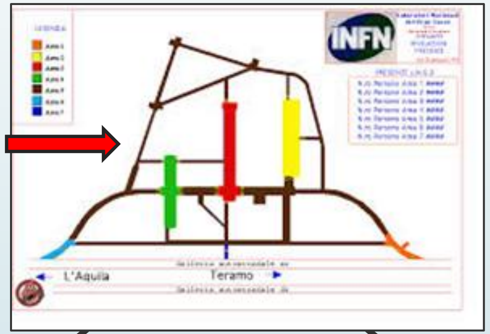
Reference Radiation Environment, RRE

SMALL ANIMALS

CELL CULTURE

Chemistry and Chemical Plant Service

Low Radiation Environment, LRE



Cosmic Silence

SMALL ANIMALS

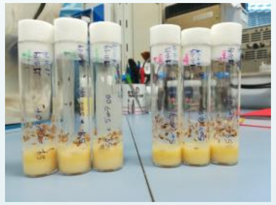
Pulex

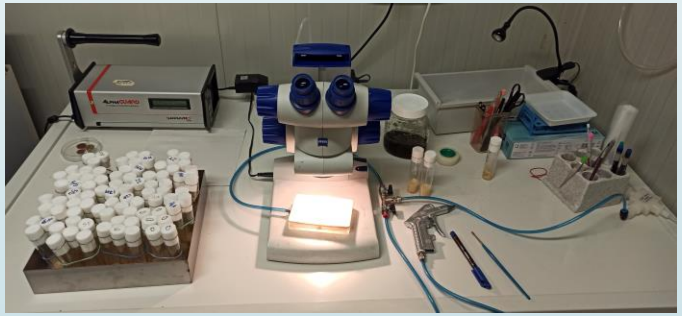
CELL CULTURE

THE LNGS UNDERGROUND BIOLOGY FACILITIES

Cosmic Silence

- 2 refrigerated incubators (for Drosophila)
- CO_2 system to manipulate Drosophila
- Stereomicroscope





Pulex

- **2 identical CO_2 incubators:** one is in a ancient iron shield (5 times reduction of gamma ray contribution)

- Biological hood
- Inverted microscope
- Refrigerator (4-6°C and -20°C)
- Thermostatic water bath
- Cryogenic storage dewar

Constant Alphaguard monitoring

RADON
Atmospheric pressure
Relative umidity

P. Morciano-Cosmic Silence Collaboration

THE LNGS ABOVE GROUND BIOLOGY FACILITY

Chemistry and Chemical Plant Service

FOR CULTURE CELL

- 1 CO_2 incubator, identical to the underground ones
- Biological hood
- Inverted microscope
- Refrigerator (4-6°C and -20°)
- Thermostatic water bath
- Cryogenic storage dewar

•FOR SMALL ANIMALS (Drosophila)

- 1 refrigerated incubator : identical to the underground one
- CO_2 system to manipulate Drosophila
- Stereomicroscope
- A 10 cm thick lead shield

Drosophila as *in vivo* model system

At LNGS, in 2016, in the framework of Centro Fermi funded ***Flyinglow experiment***, we started to employ for the first time a complex multicellular organism, the fruit fly ***Drosophila melanogaster in underground biology investigations***

Our results show:

- Reduced background radiation affects development and growth of fruit fly.
- *Drosophila* as suitable model organism for underground radiobiology experiment

(Morciano et al., Journal of Cell Physiology 2018)
(Morciano et al., Radiation Research 2018)

The RENOIR EXPERIMENT

Radiation ENvirOnment triggers bIological Responses in flies: physical and biological mechanisms

INFN-CSN5 funded experiment (2020-2022)

Two main aims:

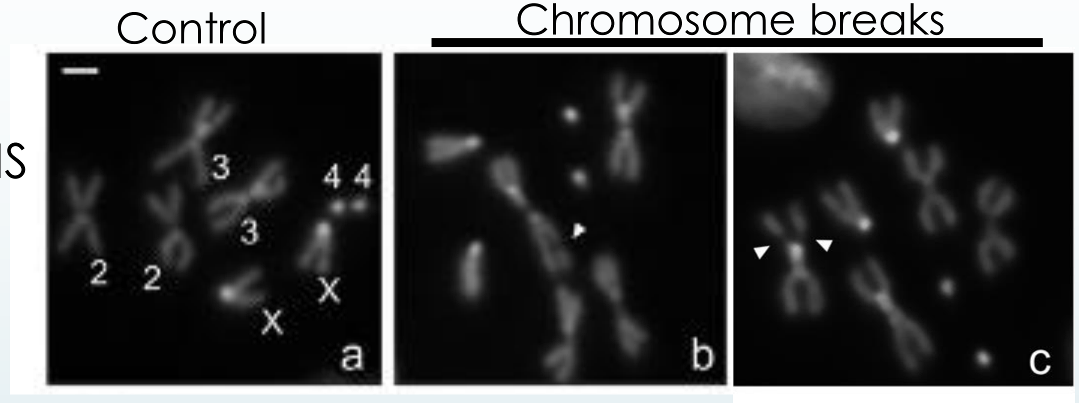
Aim 1. To improve the knowledge of the radiation field inside the external (reference) and underground laboratories, with dosimetric and spectroscopic measurements and with simulations

Aim 2. To obtain information about the involvement of the low-LET component of the radiation field on the biological responses of the fruit fly *Drosophila melanogaster*

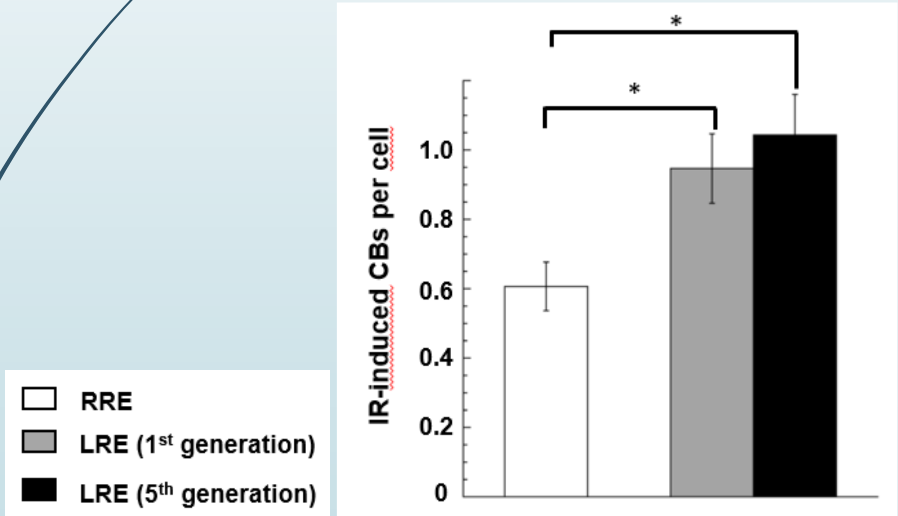
The RENOIR EXPERIMENT

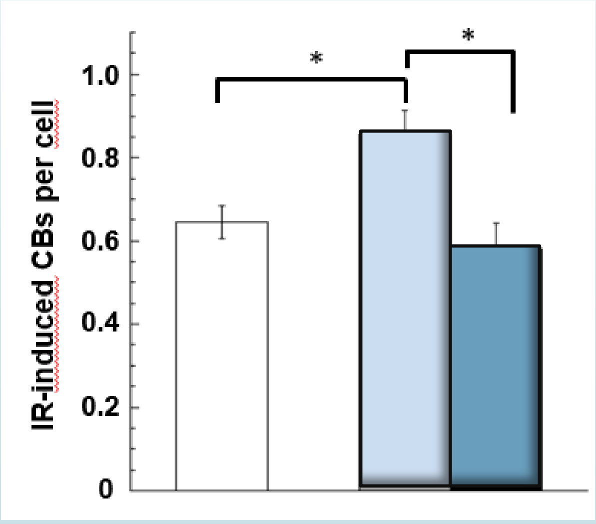
CYTOLOGICAL ANALYSIS

Larval neuroblasts



Porrazzo et a. Int J Mol Sci. 2022





Customized Marinelli beaker

Environmental radiation stimulates the DNA damage response in a complex organism

Increasing the low-LET flux (specifically of γ-rays) at LRE is able to restore the IR sensitivity of flies maintained in the reference radiation background

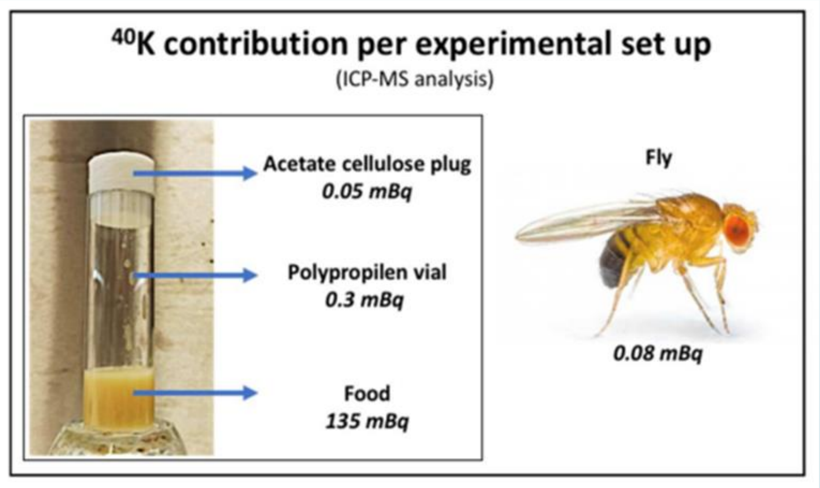
The RENOIR EXPERIMENT

•Dosimetric characterizationo of RRE and LRE

	LNGS External (RRE)	LNGS Underground (LRE)	LNGS Underground (LRE) with tuff
Photons and directly ionizing cosmic rays (low LET, mostly muons) *(nSv/h)*	47[a]	negligible	
Neutrons (high LET) *(nSv/h)*	21[b]	negligible	
Total γ-rays (terrestrial, low LET) *(nSv/h)*	31[a]	27[a]	*~90 above bk*
Total low LET dose *(nSv/h)*	78	27	~120

([a]) measures with Reuter Stokes, Automess and TLD and evaluation, at the LNGS altitude, based on UNSCEAR 2008 (Vol I. Sources and Effects of Ionizing Radiation; ([b]) literature data

•Evaluation of intrinsic radioactivity



Maximum dose rate value for intrinsic radioacrivity obtained by Monte Carlo simulation was 0.25 nGy/h (at minimum distance from the medium)

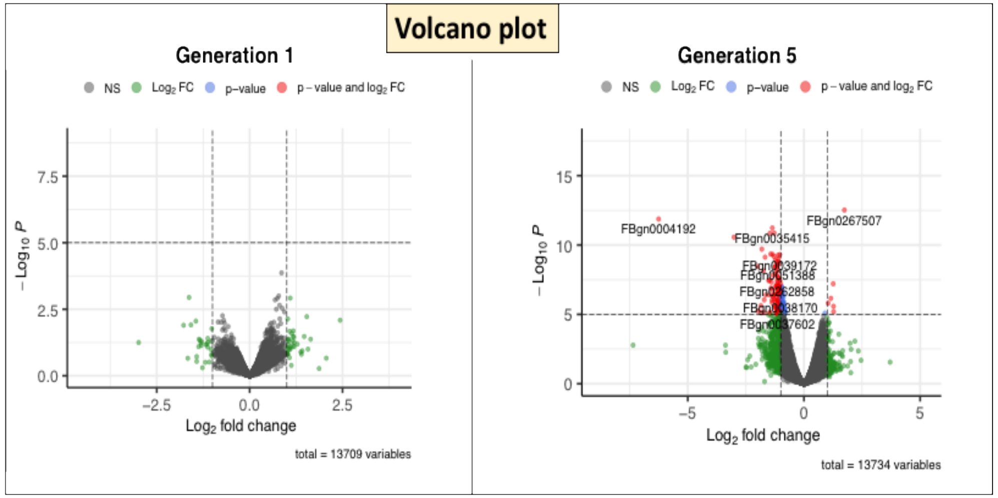
In collaboration of LNGS Special Technics (M.Laubestein) and LNGS Chemistry Service (M. Balata, S. Nisi and F. Ferella)

Article under revision in Frontiers in Physics (Special Issue Science and Technology in Deep Underground Laboratories)

The RENOIR EXPERIMENT

Comparative transcriptome analysis

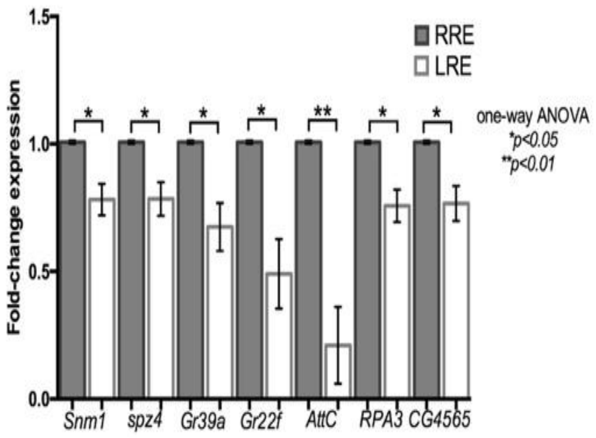
RNA SEQ analysis

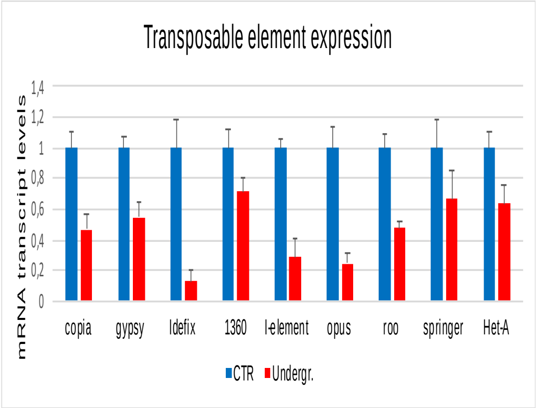


•No significant modulation in gene expression for **generation 1**

•For **generation 5,** 430 RNA transcripts (3%) significantly deregulated in LRE flies compared to RRE flies

RT-PCR validation





Genes involved in **DNA related processes** are differentially expressed (mostly down-regulated) in LRE, in agreement with the different radiation sensitivity

The manuscript is in the drafting stage

The DISCOVER22 experiment

"DNA Damage and Immune System COoperation in VEry low Radiation environment"

INFN-CSN5 funded experiment (2023-2025)

Interdisciplinary experiment divided in three work packages (WP)

WP1: Radioimmunobiology: *in vitro* studies (cells)

Task 1.1 - Immune system pathway activation

Task 1.2 - Modulation of immune system's differentiation

INFN ROMA TRE Istituto Nazionale di Fisica Nucleare Sezione di Roma Tre

INFN ROMA Istituto Nazionale di Fisica Nucleare Sezione di Roma

WP2: Radioimmunobiology: *in vivo* studies (*Drosophila melanogaster*)

Task 2.1 – Analysis of the immune system genes

INFN LNGS Istituto Nazionale di Fisica Nucleare Laboratori Nazionali del Gran Sasso

WP3: Physic studies

Task 3.1 - Microdosimetry

Task 3.2 - Biophysical modelling

INFN LNL Istituto Nazionale di Fisica Nucleare Laboratori Nazionali di Legnaro

INFN PAVIA Istituto Nazionale di Fisica Nucleare Sezione di Pavia

TIFPA Trento Institute for Fundamental Physics and Applications

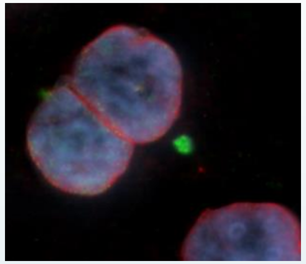
Perspective under revision in Frontiers in Physics (Special Issue Science and Technology in Deep Underground Laboratories)

WP 1: Radioimmunobiology: *in vitro* studies

Human keratinocytes will be grown in **LRE** and_**RRE** for the **0.5, 1 and 2 months.**

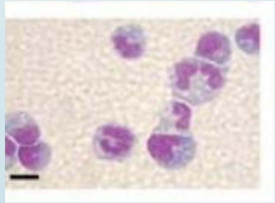
Afterwards, the cells will be **irradiated with an X-ray challenging dose of 1-2 Gy**.

DNA damage and the signal of cGAS-STING -a measure of innate immune system activation- will be analyzed.



Human promyeloblasts will be grown in **LRE** and **RRE** for the **0.5, 1 and 2 months.**
Afterwards, the cells will be induced to differentiate using chemical compounds in specialised immune system's cells : **(A) neutrophils** and (**B) macrophages**.

Differentiation and maintenance of biological function will be studied.



A

B

WP 2: Radioimmunobiology: *in vivo* studies

Drosophila melanogaster: To get information on the **expression of genes related to the immune response in fruit flies** taking advantage from the transcriptomic analysis performed in the framework of the **RENOIR experiment**.

INFN LNGS
Istituto Nazionale di Fisica Nucleare
Laboratori Nazionali del Gran Sasso

Università degli Studi dell'Aquila

WP 3: Physic studies

Task 3.1 - Microdosimetry

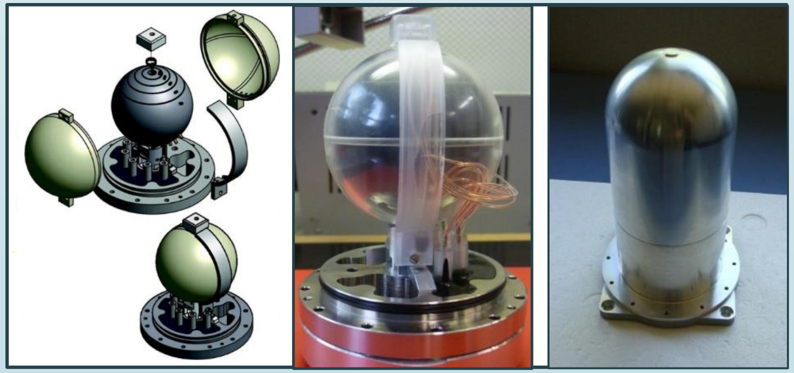
Microdosimetry: A microdosimetric monitoring of the radiation field will be performed **focused in the stochastic aspects of energy deposition by single events at low doses.**

At the documented dose levels of about **27nGy/h** in underground from RENOIR measurements, **the expected number of events in a 1 mm site is 5 10^{-7}/h.**

New detector TEPC (tissue equivalent proportional counter) will be constructed at LNL and installed in the underground laboratory (second and third year).

New detector allows to monitor both the dose and the microdosimetric spectrum on a daily base with significant statistics and will allow to identify of fluctuations in the radiation field.

.

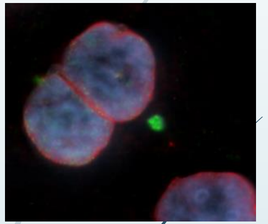


The EUTEPC of LNL. A schematic view (left)
and pictures of the internal sensor (centre)
and external case (right).

WP 3: Physic studies

Task 3.2 - Biophysical modelling

Biophysical radiation model: the link between physical **microdosimetric measures** and the corresponding **biological radiation response** will be studied employing radiation **biophysical models**.



- **Development of a biophysical model of cell cycle progression of human keratinocytes including radiation-induced DNA damage in order to identify differences** in the behaviour of LRE vs RRE-grown cells

- **Application of advanced data analysis techniques** (e.g. based on machine learning algorithms) **to identify even subtle changes**, as expected in a low-radiation background, **in the ability of immature immune cells** (promyeloblasts) **to differentiate**

An accurate model representation can help understanding response mechanisms in the biological system behaviour following exposure to below background dose rate.

On behalf of the PULEX-COSMIC SILENCE collaboration

INFN LNGS
Istituto Nazionale di Fisica Nucleare
Laboratori Nazionali del Gran Sasso

INFN ROMA
Istituto Nazionale di Fisica Nucleare
Sezione di Roma

ROMA TRE
UNIVERSITÀ DEGLI STUDI

ISTITUTO SUPERIORE DI SANITÀ

Istituto Superiore di Sanità

INFN ROMA TRE
Istituto Nazionale di Fisica Nucleare
Sezione di Roma Tre

TIFPA
Trento Institute for Fundamental Physics and Applications

Università degli studi dell'Aquila

INFN LNF
Istituto Nazionale di Fisica Nucleare

INFN PAVIA
Istituto Nazionale di Fisica Nucleare
Sezione di Pavia

External collaborations

SAPIENZA
UNIVERSITÀ DI ROMA

TOR VERGATA
UNIVERSITÀ DEGLI STUDI DI ROMA

Thank you for the attention !

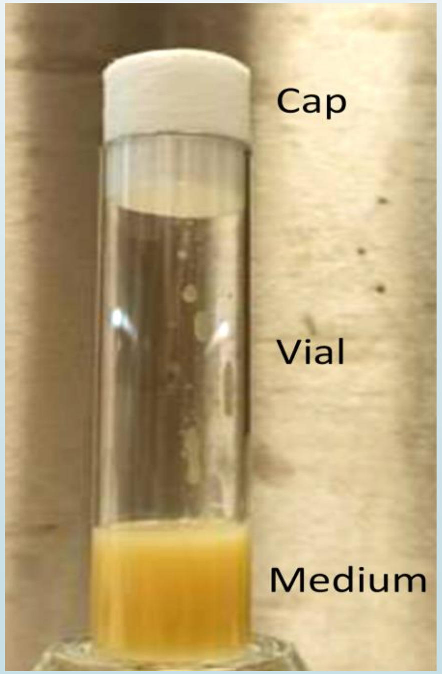
• Measurements and dose rate of intrinsic radioactivity of the setup materials

High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS)

- destructive technique
- elaborate sample processing
- shorter measurements times
- faster and more sensitive to U and Th (primordial parents)

Specific activity of fly by HR-ICP-MS

Specific activity of **caps, vials, culture medium (food)**



Gamma ray spectroscopy high pure germanium (HPGe) detectors

- conservative technique
- easy sample handling
- longer measurements times
- more sensitive to the gamma-active shorter-lived U and Th progenies (gamma emitting nuclides)

• Measurements and dose rate of intrinsic radioactivity of the setup materials

Specific acivity ^{40}K

CAP
0,05mBq/cap

VIAL
0,3 mBq/vial

MEDIUM
135 mBq/7g

fly by HR-ICP-MS
0,082 mBq/fly

Specific activity (mBq/Kg)								
		ICP-MS§				HPGe		
		Vial	Cap	Culture medium	Fly	Vial	Cap	Culture medium
^{323}Th		0,2	1,1	2,8	0,8			
	^{228}Ra					<5,8	<9,5	<23
	^{228}Th					<5,4	<15	<15
^{238}U		0,9	2,4	6,2	2,5			
	^{226}Ra					5 ±2	<5,8	<8,6
	^{234}Th					<340	<57	<210
	^{234m}Pa					<220	<250	<560
^{40}K		42	32	19*10³	93*10³	<45	<120	17,8*10³ ±1,8*10³
^{235}U						<4,7	<8	<19
^{137} Cs						<1,5	<5,9	<7
^{7}Be						30 ±10		

§ The ICP-MS analysis was performed in semi-quantitative mode
Error is estimated within 20%

- Experimental set up is ‘clean’
- Major contribution comes from **^{40}K in culture medium: 0,25 nGy/h** dose rate due to culture medium in the vial (Monte Carlo simulations)