ISIS@MACH ITALIA

ISIS Neutron and Muon Source — Science and Technology Facilities Council

Experiment Proposal

Experiment number GP2024018

Principal investigator	Dr Diego Sbardella, IRCCS Fondazione G.B. Bietti, ITALY
Co-investigator (*)	Dr Triestino Minniti, University of Rome Tor Vergata, ITALY
Co-investigator	Professor Alessio Bocedi, University of Rome, Tor Vergata, ITALY
Co-investigator	Dr Giovanni Romanelli, University of Rome Tor Vergata, ITALY
Co-investigator	Dr Laura Fazi, University of Rome Tor Vergata, ITALY
Co-investigator	Professor Roberto Senesi, University of Rome Tor Vergata, ITALY
Co-investigator	Dr Luigi Ambrosio, National Research Council, ITALY
Co-investigator	Dr Tommaso Rossi, IRCCS Fondazione Bietti ONLUS, ITALY
Co-investigator	
Experiment title	Characterisation of surgically removed human vitreous samples by second harmonic generation microscopy measurements
MRF Instrument	**Fluorescence Microscopy** — **Days requested:** 2
Access Route	Direct Access — **Previous GP Number:** GP2023048
Science Areas	Biology and Bio-materials, Medicine, Physics — **DOI:** -
Sponsored Grant	Yes — **Sponsor:** Other
Grant Title	Profiling of physical and proteomics parameters of vitreous body in retinal detachment — **Grant Number:** 5*1000 to IRCCS Fondazione Bietti
Start Date	01/03/2023 — **Finish Date:** 01/03/2025
Similar Submission?	-
Industrial Links	BVI Medical

Non-Technical Abstract

Rhegmatogenous Retinal Detachment (RD) is a severe eye disease that occurs when the retina becomes detached from the Retinal Pigment Epithelium due to the presence of retinal tears or holes. The gold standard treatment of RD is vitrectomy, that is the removal of part of the vitreous humor (VH) using vitreous cutters. A major question still unanswered, is whether there is a relation between the morphology (dimensions) of VH fragments generated by cutters when set with different frequency parameters. Here we aim at providing micro-m to mm scale characterization of the collagen content and macromolecular organization to complement microstructural characterization accesses carried out previously and submitted as continuation/repetition proposals by our team (GP2024008).

Publications

T. Rossi et al., Retina 34 (2014), 1896-904.
T. Rossi et al., Invest Ophthalmol Vis Sci. 12 (2014), 8289-94.
T. Rossi et al., Translational Vision Science & Technology 11 (2022), 29.

ISIS neutron and muon source — **E-platform:** No

Instruments	**Days Requested:**
Access Route	**Previous RB Number:**
Science Areas	**DOI:**
Sponsored Grant	**Sponsor:**
Grant Title	**Grant Number:**
Start Date	**Finish Date:**
Similar Submission?	
Industrial Links	

Sample record sheet

Principal contact Dr Triestino Minniti, University of Rome Tor Vergata, ITALY
MRF Instrument **Fluorescence Microscopy** **Days Requested:** 2
Special requirements:

SAMPLE

Material	Humor vitreous	-	-
Formula	-	-	-
Forms	Liquid		
Volume	0.002 ml		
Weight	2 mg		
Container or substrate	-	-	-
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	- K	-	-
Pressure Range	- mbar	-	-
Magnetic field range	- T	-	-
Standard equipment	-	-	-
Special equipment	-	-	-

SAFETY

Prep lab needed	Yes	-	-
Sample Prep Hazards	-	-	-
Special equip. reqs	-	-	-
Sensitivity to air	No	-	-
Sensitivity to vapour	No	-	-
Experiment Hazards	-	-	-
Equipment Hazards	-	-	-
Biological hazards	-	-	-
Radioactive Hazards	-	-	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Disposed by IS	-	-

1. **Background and Context**

Rhegmatogenous Retinal Detachment (RD) is a severe eye disease [1] that occurs when the retina becomes detached from the Retinal Pigment Epithelium (RPE) due to the presence of retinal tears or holes. The gold standard treatment of RD is vitrectomy, that is the removal of part of the vitreous humor (VH), a gel-like fluid that shapes the eye globe, using vitreous-cutters. About 15-20% of all RDs relapse within the first 6 months through Proliferative Vitreo-Retinopathy [2] (PVR), which is characterized by inflammation, collagen deposition and retinal contraction. All vitreous cutters consist of a reciprocating blade with cut-rates between 1,000 and 20,000 cuts/min. Cutters have evolved from 20G (0.9 mm out diameter in section) to 25G (0.5mm) and even 27G (0.4mm), making the internal fluidics challenging and requiring high aspiration vacuum up to 650 mmHg. High suction and blade motion applied to the collagen mesh of vitreous exert traction on the retina especially when the peripheral “vitreous base” is removed. A major question still unanswered, is whether there is a relation between intraoperative retinal traction, PVR onset and the dimensions of VH fragments (mostly collagen and proteoglycan) generated by cutters when set with different frequency parameters or whether these parameters have no effects on VH fragmentation [3]. This proposal joins the multidisciplinary research program of IRCCS Fondazione Bietti, a main Italian clinical centre for Ophthalmology, granted by the Ministry of Health (Profiling of physical and proteomics parameters of vitreous body in retinal detachment) and supported by industries. Here we focus on the non-invasive techniques for the supramolecular characterisation of vitreous material. Structural macromolecules and supramolecular organisation of the VH maintain a dilute network of collagen fibrils that are mixed in composition, with a concentration of collagen estimated to be approximately 300 mg/ml in human eyes. The predominant collagen in vitreous is type II collagen, accounting for approximately 75% of the total collagen [6].

A previous Training IM@IT access – see the experimental report GP2023073- has shown the potential of Fluorescence Microscopy for SHG measurements for the imaging of collagen in a section of tissue. The capabilities of the Fluorescence Microscopy instrumentation at the Milano Bicocca Unit have been previously reported to map the features of the collagen architecture in tissues at the μm scale in a non-invasive way [7]. Parameters such as mean fibril orientation and molecular anisotropy, along with supramolecular selectivity capability would provide relevant and complementary information in the μm to mm scales with respect to the microstructural characterization accesses carried out and submitted as continuation/repetition proposals by our team (GP2024008). Previous SEM measurements (GP2023048) on VH fragments dispersed within the saline solution have been affected by noise background (Figure 1 a)) due to the poor dilution of salt particles. This issue has now been solved during transmission electron microscopy (TEM) measurements (GP2023049) successfully performed after the SEM measurements and shown in Figure 1 b) and c).

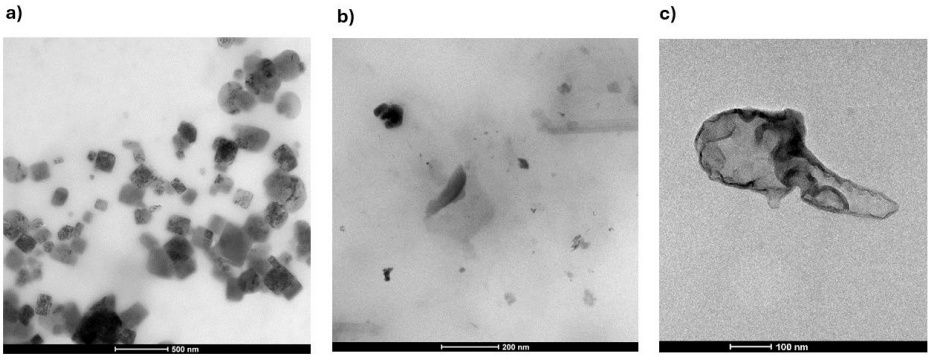
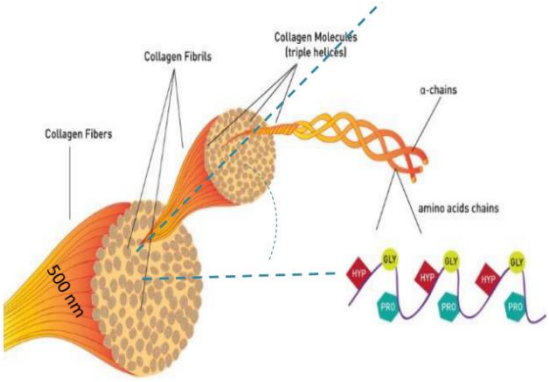


Figure 1: a) TEM image of the VH sample with poor dilution of salt particles. b) TEM image of the sample after good dilution treatment where salt particles have considerably reduced. c) TEM zoom image of a particle of VH isolated within the sample.



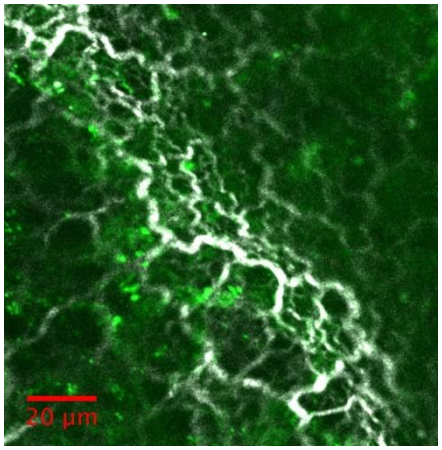


Figure 2) Multi-scale structure of collagen (left) and example of a second harmonic image of collagen in biological tissue (right- in white) - credit by Nelson Glyn.

The proposed SHG measurements will be performed on the same set of VH samples already investigated by the TEM FEI instrument operating at the IPCB-CNR Unit to complete and for direct comparisons of results and in the related proposal (GP2024008).. Documentation on the ethical issues associated to the use of VH fragments will be provided upon request.

2. Proposed experiment for second harmonic generation microscopy

In the present proposal we wish to measure the collagen distribution over up to 0.5 mm x 0.5 mm fields of view, of 6 distinct VH fragments (3 samples for each vitreous cutter frequency, i.e., 5000 CPM and 20000 CPM) using the BX51 microscope coupled to a scanning head for raster scanning biological samples. Results will be compared and integrated with the SEM images and AFM topography measured in the related proposed experiment (GP2024008), and finally compared with TEM data already collected in previous experiment (GP2023049).

3. Summary of previous experimental proposals or characterisation

The performance of vitreous cutters, by means of hydraulic resistance posed by cut VH during aspiration, has been investigated for frequencies < 12000 CPM [3,4]. Previous SEM measurements (experiment number GP2023048) on VH fragments dispersed within the saline solution have been affected by large noise background (Figure 1 a)) due to the poor dilution of salt particles. This issue has now been solved thanks to the experience in the previous SEM measurements and by transmission electron microscopy (TEM) measurements (GP2023049) successfully performed after the SEM measurements and shown in Figure 1 b) and c). Moreover, X-ray computed tomography (XCT) measurements (GP2023050) have been carried out to reveal the 3D morphology of VH fragments surgically isolated from RD patients, and analysis on these data are in progress.

4. Justification of experimental time requested for SEM

VH fragments (6 in total, 3 samples for each vitreous cutter frequency, i.e., 5000 CPM and 20000 CPM) will be measured by SHG scans. We predict at least n. 8 images per sample. Hence, after the experience gained by previous experiments and training accesses, we request 2 days of instrument time including set-up and calibration time.

5. References

[1] T. Schick et al., Klin Monbl Augenheilkd. 12 (2020), pp. 1479-1491; [2] S. Yang et al., Discov Med. 110 (2015), 207; [3] T. Rossi et al., Retina 34 (2014), 1896-904; [4] T. Rossi et al., Invest Ophthalmol Vis Sci. 12 (2014), 8289-94; [5] S. Pastor-Idoate et al., PLoS ONE 12 (2017), e0173883; [6] P. Bishop, Progress in Retinal and Eye Research 19 (2000), 323; [7] F. Radaelli et al., Scientific Reports 7 (2017) 17468.