ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

# Experiment Proposal

Experiment number GP2024008

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|---|---|---|
| **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 vitreous humor samples by SEM measurements | |
| **MRF Instrument** | **SEM with correlative AFM** | **Days requested:** 3 |
| **Access Route** | Direct Access | **Previous GP Number:** Yes (experiment 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. The proponent aim to complete by scanning electron microscopy the study on the morphology of VH fragments surgically isolated from RD patients which have been already measured by means of TEM measurements (experiment number GP2023049). In the present proposal we wish to measure 6 distinct VH fragments (3 samples for each vitreous cutter frequency, i.e. 5000 CPM and 20000 CPM) by the use of the SEM with correlative AFM instrument operating at the University of Rome Tor Vergata.

**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.

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**E-platform:** No

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|---|---|
| **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** **SEM with correlative AFM** **Days Requested:** 3
**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 a process called Proliferative Vitreo-Retinopathy [2] (PVR), which is characterized by inflammation, collagen deposition and retinal contraction. PVR is highly invalidating and often accompanied by sight loss, thus carrying a huge burden for the quality of life and for social and economic costs. All vitreous cutters base on the mechanism of a reciprocating blade moving within a hollow cylinder in a proximal-to-distal fashion, with cut-rates comprised between 1,000 and 20,000 cuts per minute. Given the miniaturization of retinal surgery instrumentation, 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 even more challenging and requiring high aspiration vacuum up to 650 mmHg to win the hydraulic resistance of the highly viscous human vitreous material. 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 and more so when the retina is mobile during retinal detachment surgery. For this reason, the intraoperative creation of iatrogenic retinal tears and the amount of traction exerted on the retina causing further damage and possibly giving rise to Proliferative Vitreoretinopathy remains and important issue, largely unresolved. A major question still unanswered, is whether there is a relation between intraoperative retinal traction, PVR onset and the morphology (dimensions) of VH fragments (mostly collagen and proteoglycan) generated by cutters when set with different frequency (cuts per minute, CPM) parameters or whether these parameters have no effects on VH fragmentation [3]. The contribution of turbulent vitreous fluidics at the cutter port to the consistency of vitreous fragment dimensions is also a matter of speculation. If different fragments are produced, then the tensile force generated over the retina layer (which adheres to VH) as well as the mechanical stress - responsible for intra-operative retinal traction and iatrogenic break formation - may be influenced by cutter parameters. This proposal fits into a multidisciplinary research program of IRCCS Fondazione Bietti (IFB) and supported by industries. The proponents aim to repeat scanning electron microscopy (SEM-AFM) measurements of VH fragments surgically isolated from RD patients and its protein composition that can help predicting the proportion of those patients who most likely will develop PVR. Previous SEM measurements (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 lesson learned by 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). To this end, we will use SEM with correlative AFM instrument operating at UTOV Unit where the first round of measurements has been performed to analyse the same set of VH samples already investigated by the TEM FEI instrument operating at the IPCB-CNR Unit, to complete comparisons of results. The VH fragments, generated by vitreous cutters used at two frequencies, i.e., 5000 and 20000 CPM, will be isolated from the same patient eye during two surgical phases, using an established surgical procedure and Good Medical Practices [3]. Ethical issues associated to the VH fragments will be provided upon request.

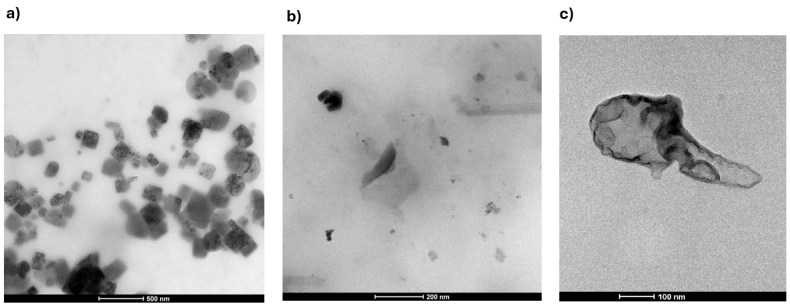


*Figure 1: a) TEM image of the VH sample with poor dilution of salt particles. b) TEM image of the sample after good dilution treatment where salt particles have considerably reduced. c) TEM zoom image of a particle of VH isolated within the sample.*

## 2. Proposed experiment for SEM

In the present proposal we wish to measure the morphology and topography feature of 6 distinct VH fragments (3 samples for each vitreous cutter frequency, i.e., 5000 CPM and 20000 CPM) using the SEM with correlative AFM instrument. Results from SEM images (mean size and standard deviation of each macromolecular fragments in VH) and AFM topography (EDX used to identify collagen fibrils) measured in this experiment will be compared with TEM data already collected in previous experiment (experiment number GP2023049).

## 3. Summary of previous experimental proposals or characterisation

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 lesson learned by the previous SEM measurements and by transmission electron microscopy (TEM) measurements (experiment number GP2023049) successfully performed after the SEM measurements and shown in Figure 1 b) and c). Moreover, X-ray computed tomography (XCT) measurements (experiment number GP2023050) have been done 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 SEM, EDX and AFM scans using a field of view and magnification which depends on the macromolecular fragments in VH. We predict n. 8 images per sample and few AFM topography images. Hence, after the experience gained by previous experiment, we request 3 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.