GRAPHENE OXIDE DETECTION IN AQUEOUS SUSPENSION

OBSERVATIONAL STUDY IN OPTICAL AND ELECTRON MICROSCOPY

Interim report (I)

June 28, 2021

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Unauthorized English translation. Original: https://www.docdroid.net/rNgtxyh/microscopia-de-vial-corminaty-dr-campra-firma-e-1-fusionado-pdf

IMPORTANT ANNOUNCEMENT
Next, a microscopic, observational and merely descriptive study of the test sample is presented.
Definitive identification of the dominant material in the sample requires further fractionation and specific spectroscopic analysis to characterize the structure of the material.

Background

• **Mr. Ricardo Delgado Martín** requests PROVISION OF RESEARCH SERVICES to the UAL named:

"DETECTION OF GRAPHENE IN AQUEOUS SUSPENSION SAMPLE"

- On 06/10/2021 1 vial was received by courier, labeled with the following text:

- “COMIRNATY™ .Sterile concentrate. COVID-19 mRNA. 6 doses after dilution.
- Discard date / time: PAA165994.LOT / EXP: EY3014 08/2021 ”

- Origin and traceability: unknown
- State of conservation: refrigerated
- Maintenance during the study: refrigerated
- Coding of the problem sample to be analyzed: RD1

Preliminary observations of the test sample RD1

Description:
- Sealed vial, with rubber and aluminum cap intact, of 2 ml capacity, containing a 0.45 ml cloudy aqueous suspension.
- RNA extraction and quantification is performed

- Presence of uncharacterized nanometric microbiology is observed, visible at 600X in optical microscope

Sample processing

1. Dilution in 0.9% sterile physiological saline (0.45 ml + 1.2 ml)
2. Polarity fractionation: 1.2 ml hexane + 120 ul of RD1 sample 3. Extraction of hydrophilic phase
4. Extraction and quantification of RNA in the sample
5. Electron and optical microscopy of aqueous phase

Preliminary analysis: extraction and quantification of Rna in the sample

1. RNA extraction: Kit https://www.fishersci.es/shop/products/ambion-purelink-rna-mini-kit-7/10307963
2. Quantification of total UV absorbance in spectrophotometer NanoDrop™ https://www.thermofisher.com/order/catalog/product/ND-2000#/ND-2000
3. Specific quantification of Rna by fluorescence QUBIT2.0: https://www.thermofisher.com/es/es/home/references/newsletters-and-journals/bioprobes-journal-of-cell-biology-applications/bioprobes-issues-2011/bioprobes-64-april-2011/the-qubit-2-0-fluorometer-april- 2011.html

UV absorption spectrum of the aqueous phase of the RD1 sample (Nano-drop team)

Maximum absorption of SAMPLE RD1 (260-270 nm)

- RNA. It presents usual maximums at 260 nm. Total concentration estimated by QUBIT2.0 fluorometry: **6 ng / ul**
- The spectrum reveals the presence of a high quantity of substances or substances other than Rna with maximum absorption in the same region, with a total estimated at **747 ng / ul** (uncalibrated estimate)
- Reduced graphene oxide (RGO) has absorption maxima at 270 nm, **compatible** with the spectrum obtained *(Thema et al, 2013. Journal of Chemistry ID 150536)*
- **The maximum absorption obtained DOES NOT ALLOW TO DISCARD the presence of graphene in the sample. The minimum amount of RNA detected by QUBIT2.0 only explains a residual percentage of the total UV absorption of the sample.**

OBJECTIVE: Microscopic identification of graphene derivatives

METHODOLOGY:
1. Imaging in optical and electron microscopy
2. Comparison with literature images and reduced graphene oxide standard sample

TRANSMISSION ELECTRON MICROSCOPY (TEM)

Electron microscope JEM-2100Plus
Voltage: 200 kV
Resolution 0.14 nm
Magnification up to x1,200,000

TRANSMISSION ELECTRON MICROSCOPY (TEM)

Electron microscopy (TEM) is commonly used to image graphene nanomaterials. It has become a fairly standard and easy to use instrument that is capable of imaging individual layered graphene sheets.

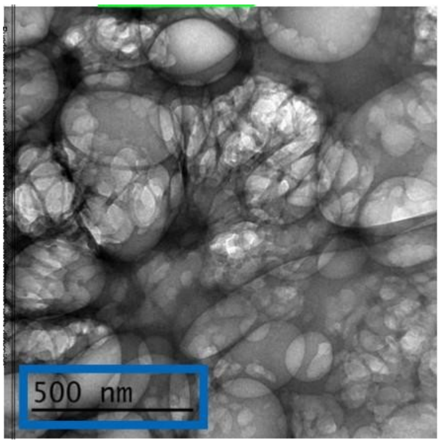
DESCRIPTION OF THE PREVIOUS IMAGE

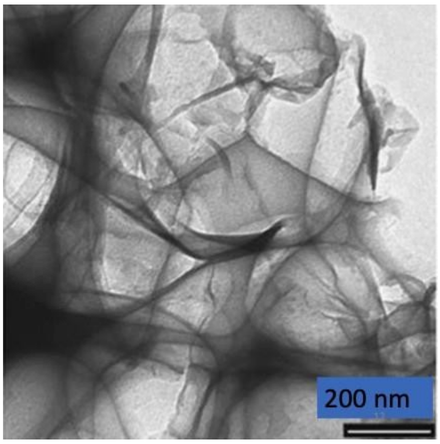
(from: Choucair et al, 2009. Gram-scale production of graphene based on solvothermal synthesis and sonication. Nature Nanotechnology 4 (1): 30-3

• Figure 2: “TEM images of the **agglomerated graphene sheets**. The same sample region is viewed at different magnifications and clearly shows the degree of sheet formation and the **tendency of sheets to fuse into overlapping regions**. An inherent sheet-like structure is evident showing **an intricate array of long-range pleats**. Since the images are taken in transmission mode, the relative opacity of the individual sheets is the result of interfacial regions with overlap between individual sheets. The sheets extend in lateral dimensions on micrometer length scales, ranging from 100 nm to more than 1,000 nm. "

RESULTS: Comparison of problem sample (RD1) with a TEM image of literature

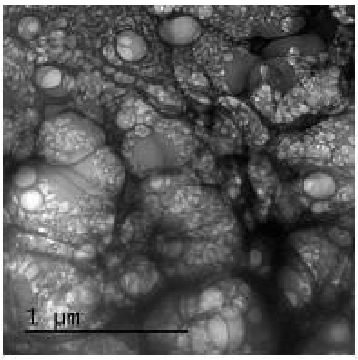
SAMPLE RD1 Choucair et al 2009. Nature Nanotechnology 4 (1): 30-3 Fig 2

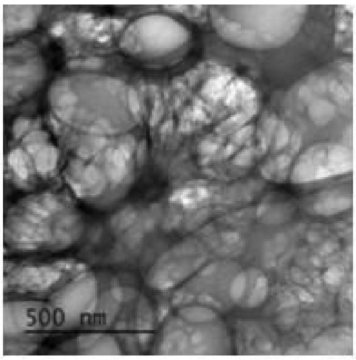


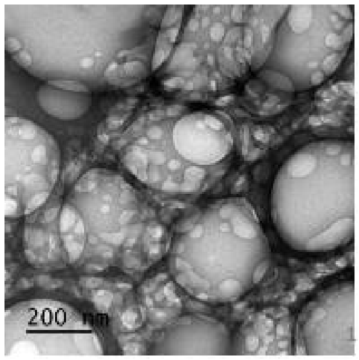


RESULTS: DESCRIPTION OF THE TEM IMAGES OF THE RD1 PROBLEM SAMPLE

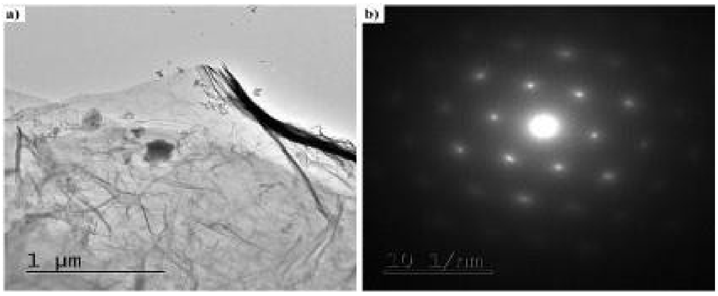
The TEM images of the RD1 sample **in general PRESENT A HIGH SIMILARITY** with images of **graphene oxide** from the literature obtained by the same TEM technique, with similar magnifications. An intricate **matrix** or **mesh of folded translucent flexible sheets** can be observed, with a mixture of darker multilayer agglomerations and lighter colored unfolded monolayers. Darker linear areas appear due to local overlap of sheets and local arrangement of individual sheets in parallel to the electron beam. After the mesh, a high density of **unidentified rounded and elliptical clear shapes** appears, possibly corresponding to holes generated by mechanical forcing of the mesh during treatment. We show here 3 images with progressive magnification:







- **Important NOTE:** For a definitive IDENTIFICATION of GRAPHENE by TEM, it is necessary to complement the observation with the structural characterization by obtaining by EDS a characteristic ELECTRON DIFFRACTION STANDARD SAMPLE (as the figure b shown below). The standard sample corresponding to graphite or graphene has a hexagonal symmetry, and generally has several concentric hexagons. **It has not been possible at the moment to obtain this standard sample due to the shortage of sample available for processing, and the chaotic arrangement and density of the folds.**



Matéria (Rio J.) 23 (1) • 2018 • Characterization of graphene nanosheets obtained by a modified Hummer's method. Renata Hack et al.

Optical microscope

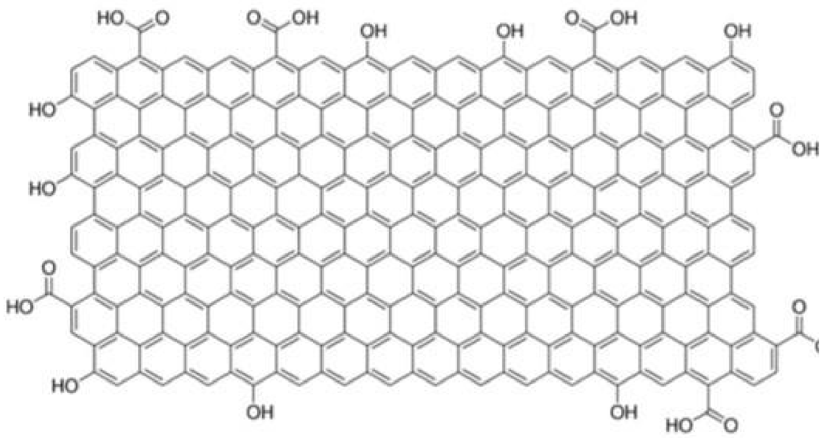
CX43 Biological Microscope

10x, 20x (DIC) and 40x (DIC) PLAN Fluor objectives

Eyepiece: 10x

• Condenser set in intermediate position with 3D effect (between light field (BF) and dark field (DF)

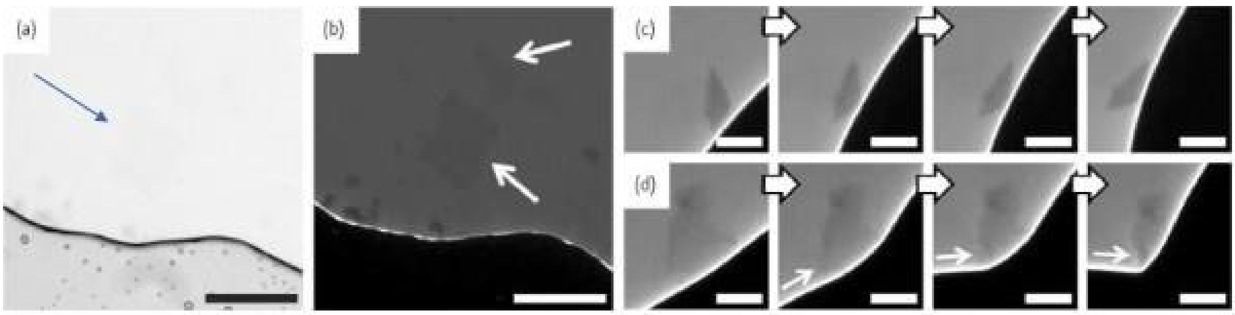
REDUCED GRAPHENE OXIDE STANDARD SAMPLE.



IDENTIFICATION OF GRAPHENE OXIDE AND ITS STRUCTURAL CHARACTERISTICS BY OPTICAL MICROSCOPY

Graphene materials essentially consist of a single atomic layer. This makes absorbance-based light microscope observation difficult, although it is possible to acquire optical images of suspended graphene sheets under brightfield transmitted light (Fig. A). Oxidized graphene (GO) has a much paler color than reduced graphene (rGO).

However, under **reflective illumination**, **high-contrast optical imaging of graphene** and even GO sheets has been reported in literature. Modifying the angle of incidence of the illumination, by means of appropriate adjustment of the condenser (bright field and dark field), this has been the technique used to increase the contrast in sample RD1 of this report and obtain images of the roughness on the surface of the sheets. with 3D effect.

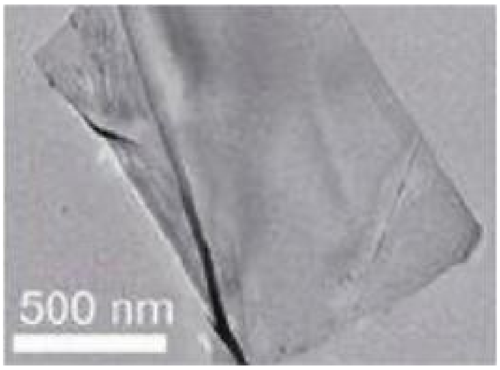


a) Bright field. b-d) Fluorescence extinction microscopy (FQM)
Kim et al, 2010. Seeing graphene-based sheets, Materials Today, Volume 13, 2010, Pages 28-38,

Literature Image
Low magnification TEM

"The figure shows a TEM image of bilayer graphene with edges that tend to curl and bend slightly"

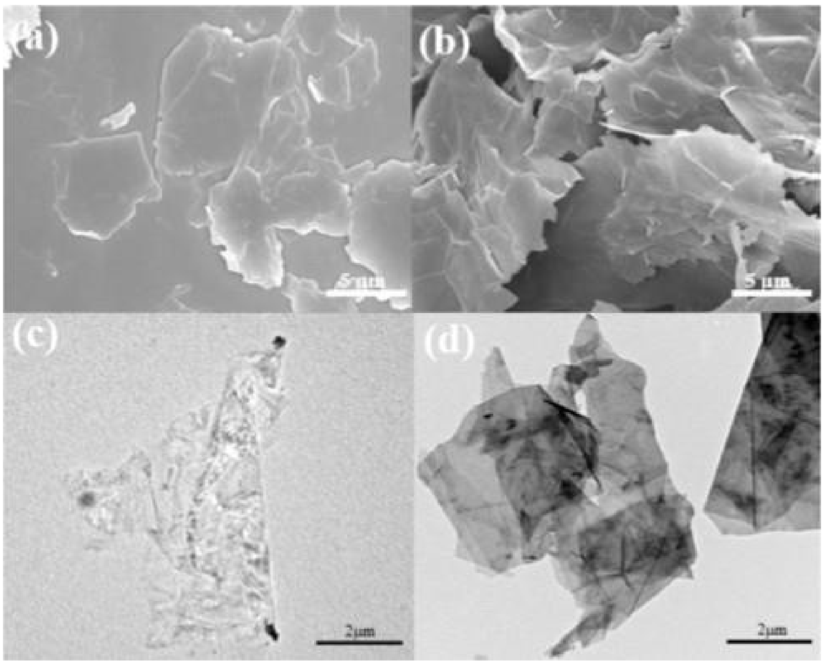
Qian, W., Hao, R., Hou, Y. et al. Solvothermal-assisted exfoliation process to produce graphene with high yield and high quality. Nano Res. 2, 706-712 (2009).



LITERATURE IMAGES. ELECTRON MICROSCOPY AT LOW MAGNIFICATION ELECTRON SCANNING MICROSCOPY (SEM) (a) and (b) and TRANSMISSION (TEM) (c) and (d)

Effects of Graphene Nanosheets with Different Lateral Sizes as Conductive Additives on the Electrochemical Performance of LiNi0.5Co0.2Mn0.3O2 Cathode Materials for Li Ion Batteries. Figure 2. SEM images of different graphene sheet sizes: (**a**) GN-13 and (**b**) GN-28, and transmission electron microscopy (**TEM**) images of different graphene sheet sizes: (**c**) GN-13 and (**d**) GN -28.

Husu et al. Polymers 2020, 12 (5), 1162



Robust Magnetized Graphene Oxide Platform for In Situ Peptide Synthesis and FRET-Based Protease Detection

Kim et al, Sensors **2020**, 20 (18), 5275

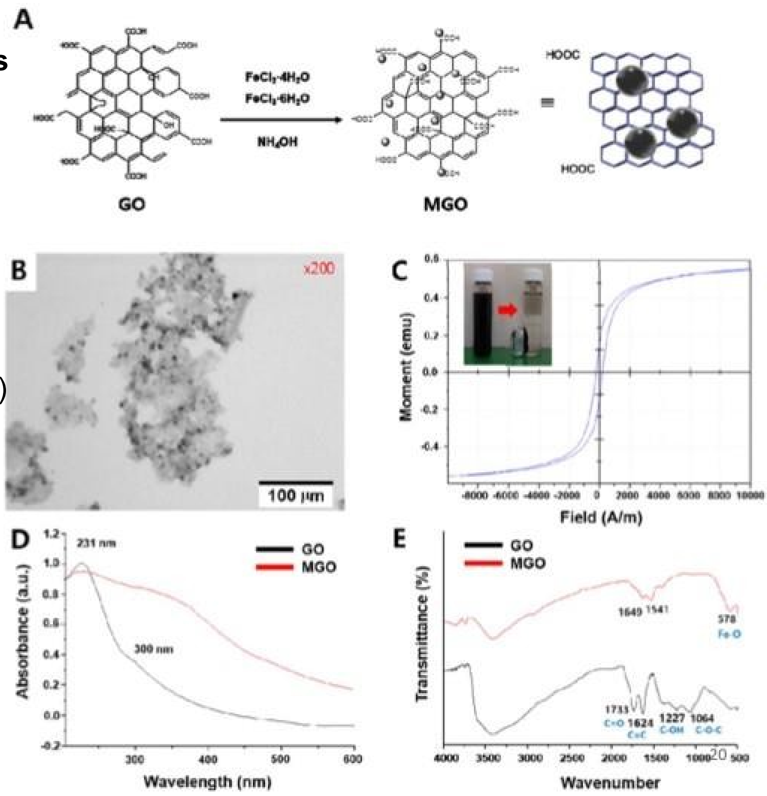
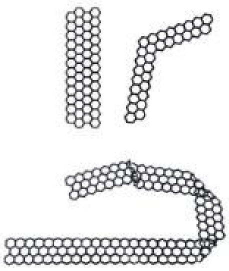


Figure 1. Preparation and characterization of graphene oxide (GO) and magnetic graphene oxide (MGO). (A) Schematic of MGO synthesis procedure. (B) Optical microscopy image of MGO. (C) Magnetic hysteresis loop of MGO. (D) UV / Vis absorption spectra of GO and MGO. (E) FT- IR spectra of GO and MGO.

Comparison of RD1 sample to the light microscope with images of the REDUCED GRAPHENE OXIDE (rGO) standard sample

The optical images of the sheets present in the RD1 sample reveal great similarity with the sheets exfoliated from sonication of the rGO standard sample. Both samples present internally rough translucent sheets, with irregular profiles, folded on themselves and with a tendency to roll up at the edges. The shapes and dimensions of the sheets are highly variable, with both samples presenting sheets in ribbons or bands folded on themselves (ribbons).

The attached ANNEX shows alternate images of STANDARD SAMPLE OF rGO and SAMPLE PROBLEM RD1



https://cen.acs.org/articles/86/i4/Graphene-Ribbons.html

CONCLUSIONS AND RECOMMENDATIONS

1. Microscopic study of the sample provides **strong evidence for the probable presence of graphene derivatives, although microscopy does not provide conclusive evidence.** The definitive identification of graphene, oxidized graphene (GO) or reduced oxidized graphene (rGO) in the RD1 sample requires the **STRUCTURAL CHARACTERIZATION** through the analysis of specific spectral standard sample comparable to those published in literature and those obtained from the standard sample, obtained with spectroscopic techniques such as XPS, EDS, NMR, FTIR or Raman, among others.

2. The analyzes in this report correspond to **ONE SINGLE SAMPLE, limited in total volume available for processing.** It is therefore necessary to carry out a significant sampling of similar vials to draw conclusions that can be generalized to comparable samples, recording origin, traceability and quality control during storage and transport prior to analysis.

Disclaimer

- **The results and conclusions of this report do not imply any institutional position of the University of Almería**
- Neither the Principal Investigator nor the University of Almería assume any responsibility for the contents and opinions of third parties regarding this report from its possible dissemination on social networks or the media, nor for the conclusions that may be drawn from it that have not been been made explicit in the text.

SEE APPENDIX PHOTOGRAPHS OF THE SAMPLE

GRAPHENE OXIDE DETECTION IN AQUEOUS SUSPENSION (COMIRNATY™ (RD1)

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PHOTOGRAPHS APPENDIX

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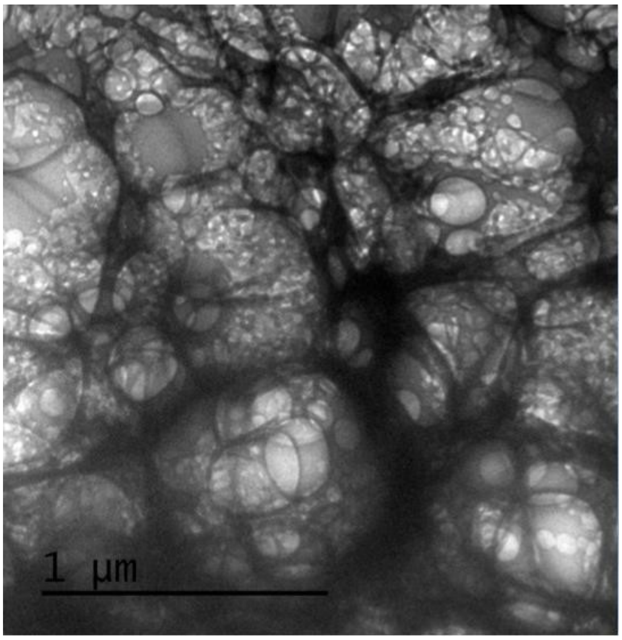
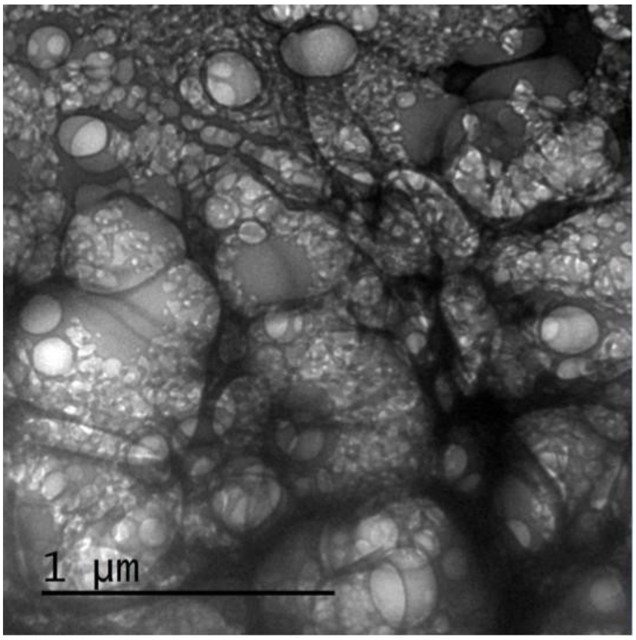
ELECTRON MICROSCOPY

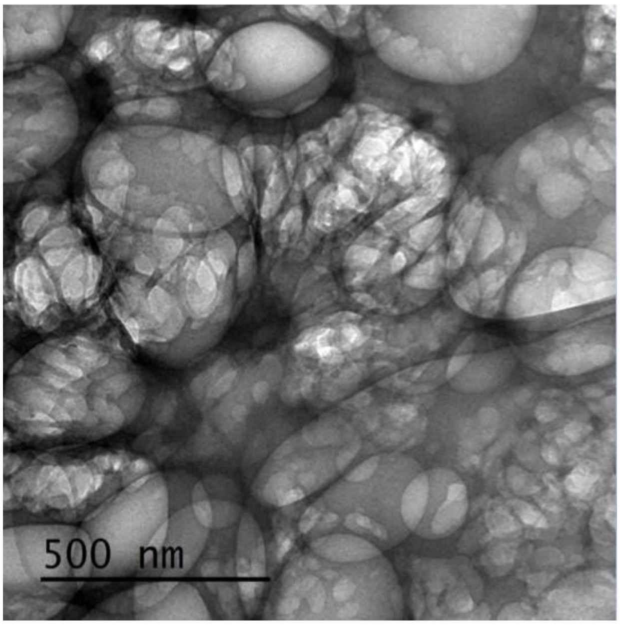
SAMPLE RD1



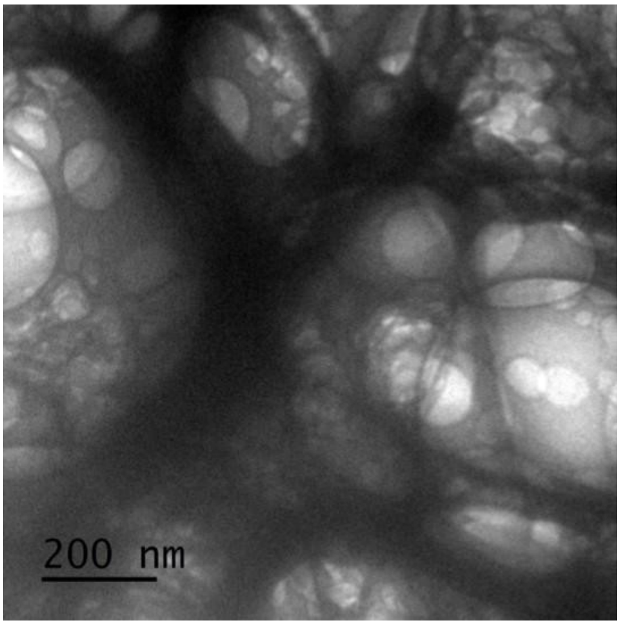
SAMPLE RD1



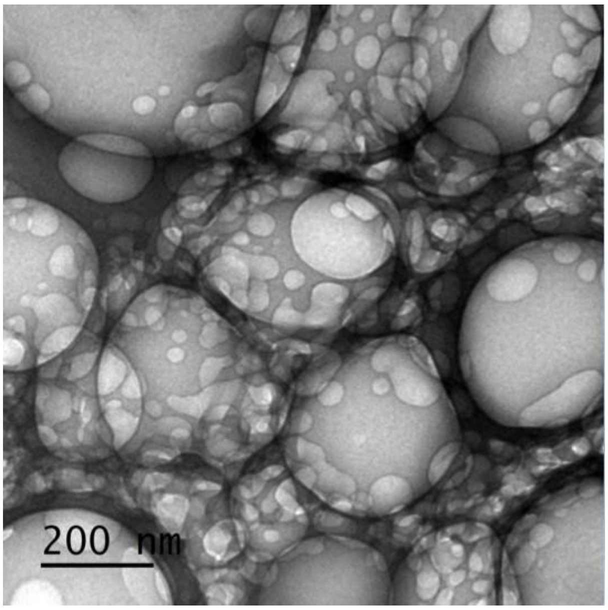
SAMPLE RD1



SAMPLE RD1

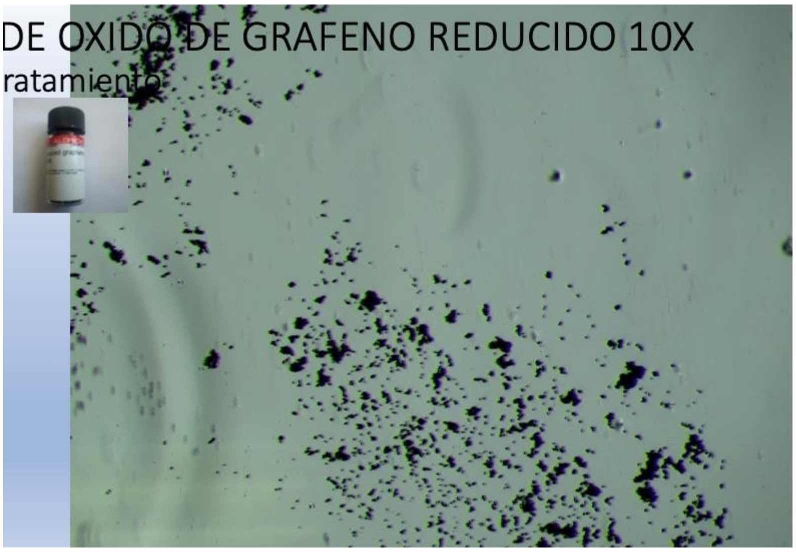


SAMPLE RD1



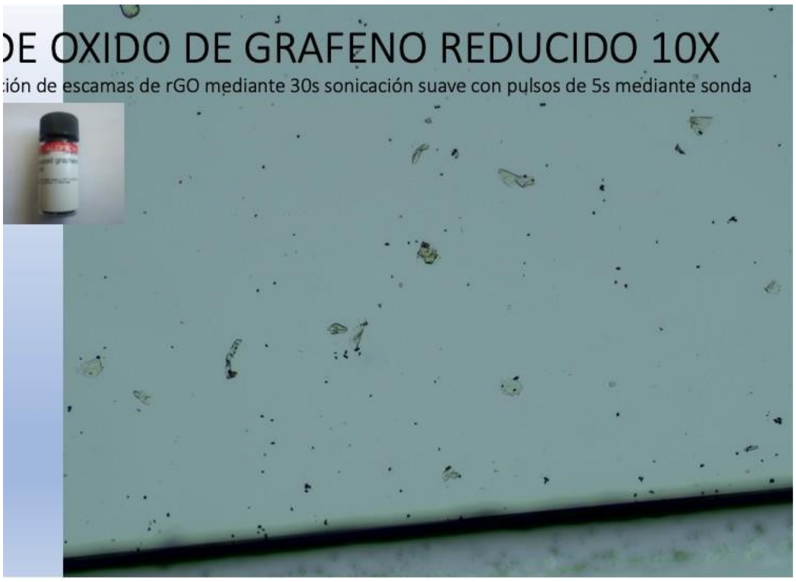
OPTICAL MICROSCOPY

10X REDUCED GRAPHENE OXIDE STANDARD SAMPLE
Before treatment

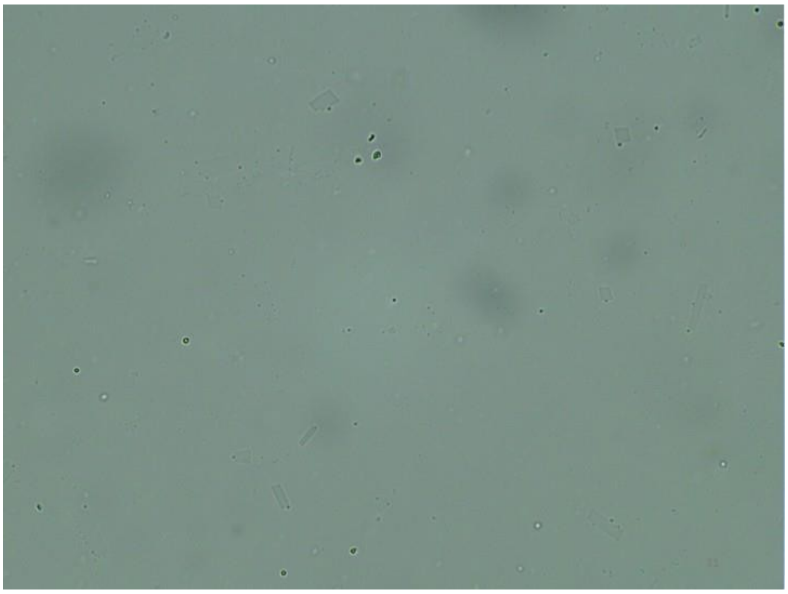


10X REDUCED GRAPHENE OXIDE STANDARD SAMPLE

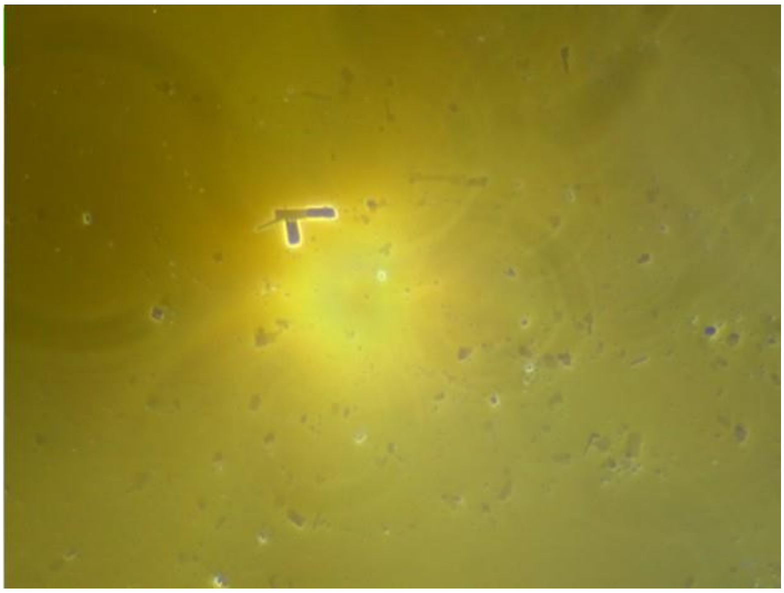
Treatment: Exfoliation of rGO scales by 30s gentle sonication with 5s pulses by probe



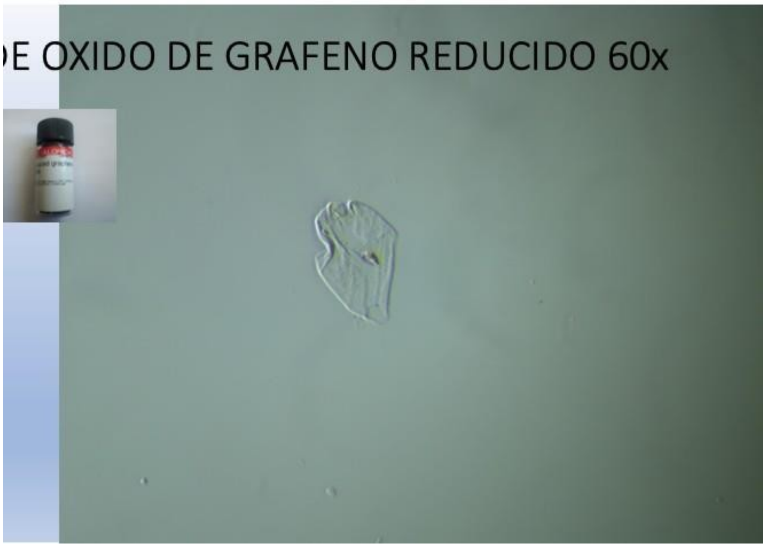
SAMPLE RD1



SAMPLE RD1 (dark field)

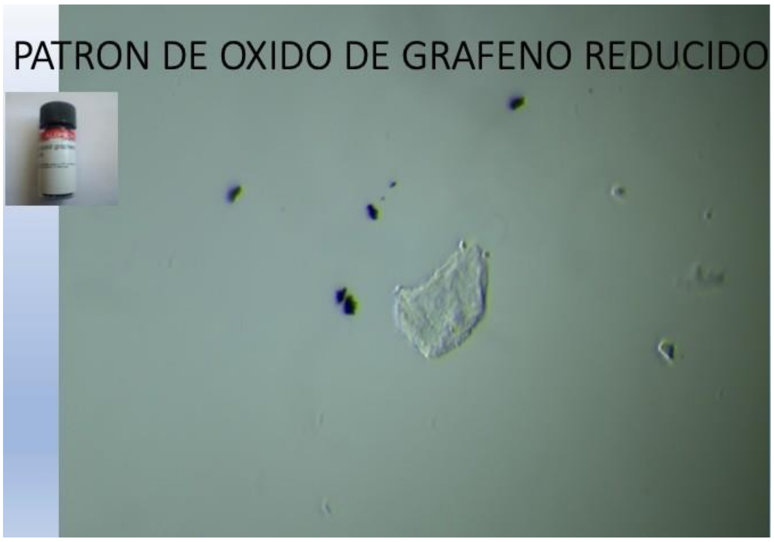


60x REDUCED GRAPHENE
OXIDE STANDARD SAMPLE

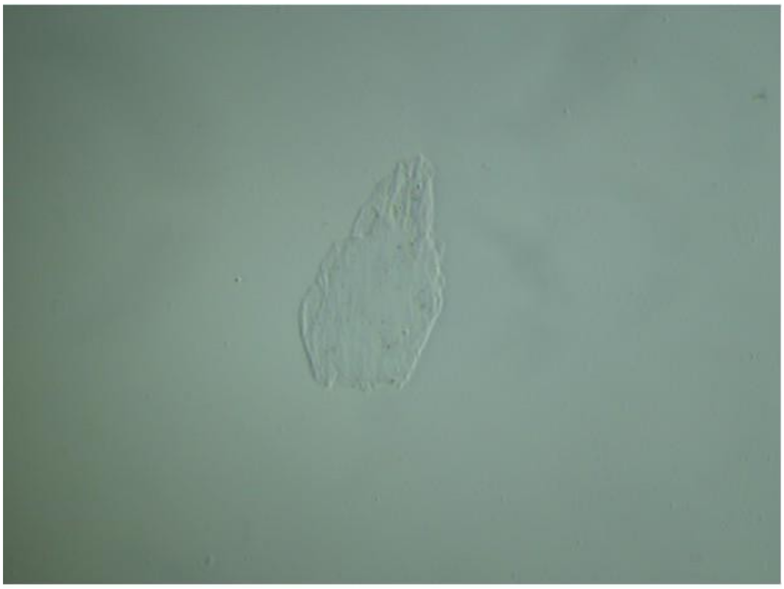


SAMPLE RD1 60x

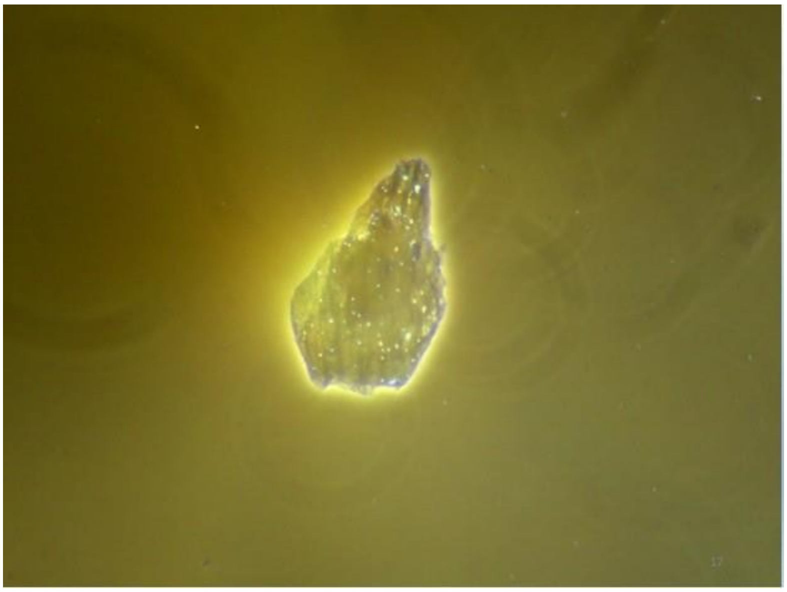
REDUCED GRAPHENE OXIDE
STANDARD SAMPLE



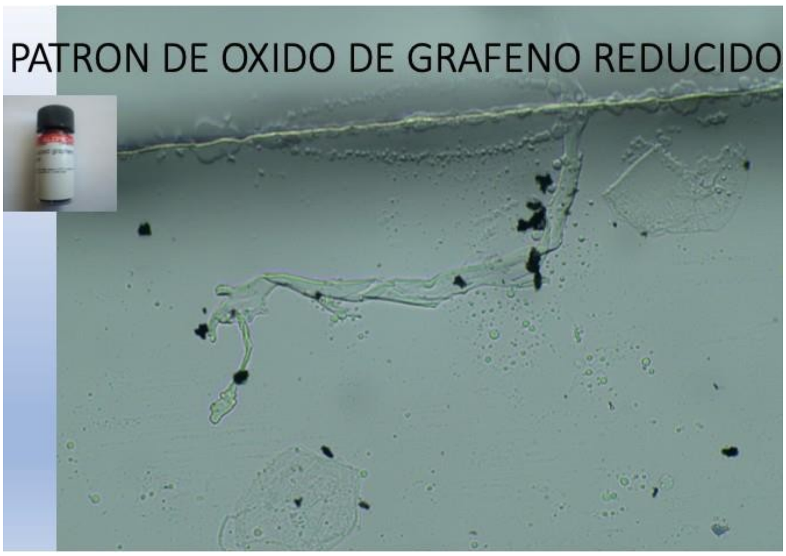
SAMPLE RD1



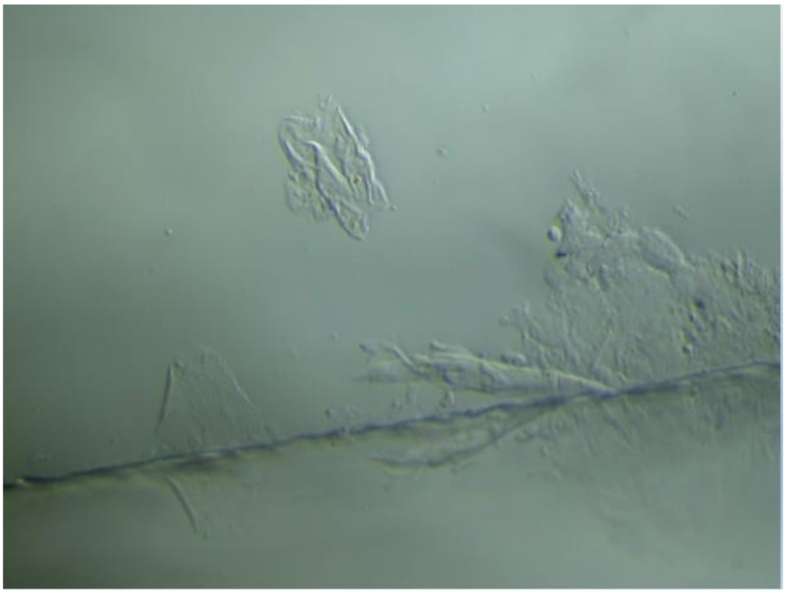
SAMPLE RD1 (dark field)



REDUCED GRAPHENE
OXIDE STANDARD SAMPLE



SAMPLE RD1



REDUCED GRAPHENE OXIDE
STANDARD SAMPLE

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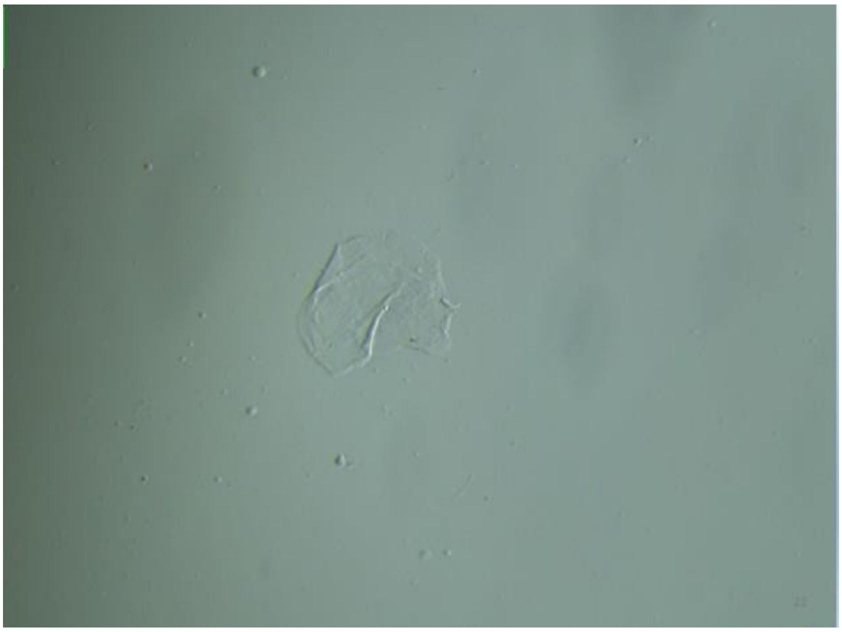
STANDARD SAMPLE

SAMPLE RD1



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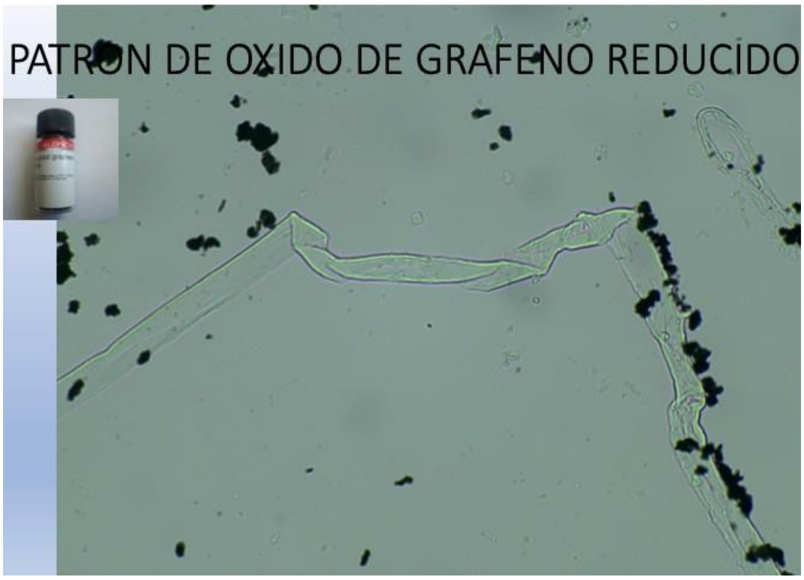
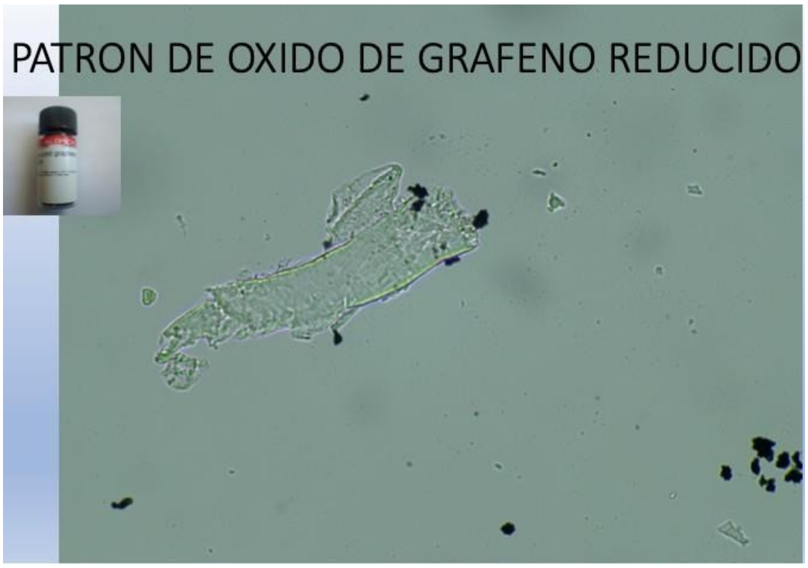


REDUCED GRAPHENE OXIDE
STANDARD SAMPLE

SAMPLE RD1

REDUCED GRAPHENE OXIDE
STANDARD SAMPLE



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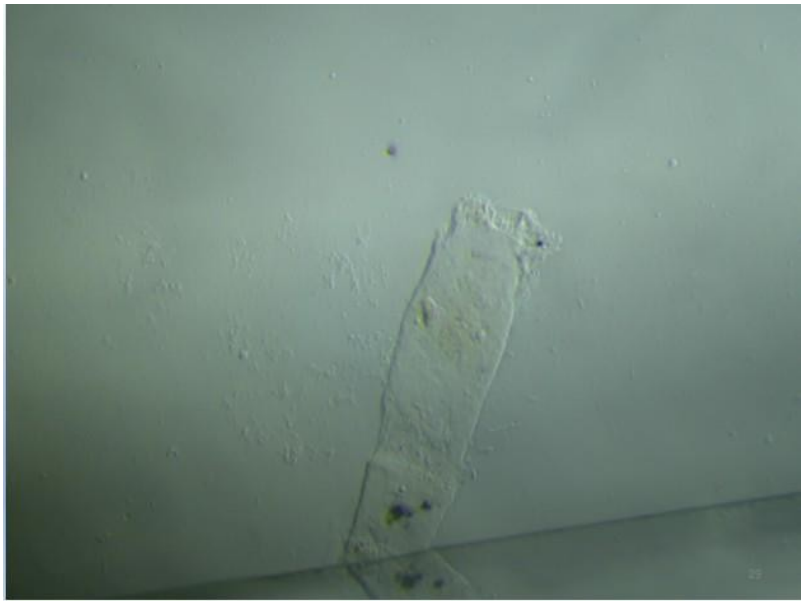

SAMPLE RD1