OPTICAL-ELECTRON MICROSCOPY ANALYSIS AND EDX CHARACTERIZATION DETECTION OF GRAPHENE NANOPARTICLES (OR DERIVATIVES) IN VIALS

METHODOLOGY

Sample reception:
Samples of graphene nanostructured solutions were received in Eppendorf tube.

Sample codes: Al-3, SI, P1, and P2 (*)

Number of samples: 6

Visual inspection: samples A translucent solution, samples P and S opalescent solution,
approx. 0.5-1 mL of each sample.

Sample processing:
A resolution test is performed.
-CONFOCAL, direct samples visualized, autofluorescence is recorded.
-SEM, representative samples (direct and diluted 1/10, 1/20, and 1/50) were mounted on an aluminum stub, and copper was delimited using silver paint.
-STEM sample of 1/20 and 1/50 dilutions for mounting on a carbon-coated copper grid. For this, a drop of the dilutions on the grid. Allowed to dry at room temperature.

Scanning Electron Microscopy Visualization
The samples were visualized by Scanning Electron Microscopy -SEM, SU3S00 HITACHI (Japan), under the following parameters: 10KV, WD 10 mm approx., using SE and BSE detector.
For elemental distribution microanalysis, the samples were analyzed under the following parameters: 15 KV, WD 10 mm approx., BSE detector coupled to EDX.

Visualization Confocal Laser Microscopy (CLSM)-Screening of structures
10 µL of the concentrated sample are mounted on slides and visualized by CLSM, registering autofluorescence at different wavelengths A emission/excitation 488nm/530nm, objective 40X zoom 2X.

(*) NOTE: Letter A corresponds to Astrazeneca ChAdOx1-S vaccine vials, P to Pfizer Comirnaty vaccine, and S to Sinovac CoronaVac vaccine.

RESULTS

CONFOCAL LASER MICROSCOPY

A3 (direct)

P2 (direct)

S1 (direct)

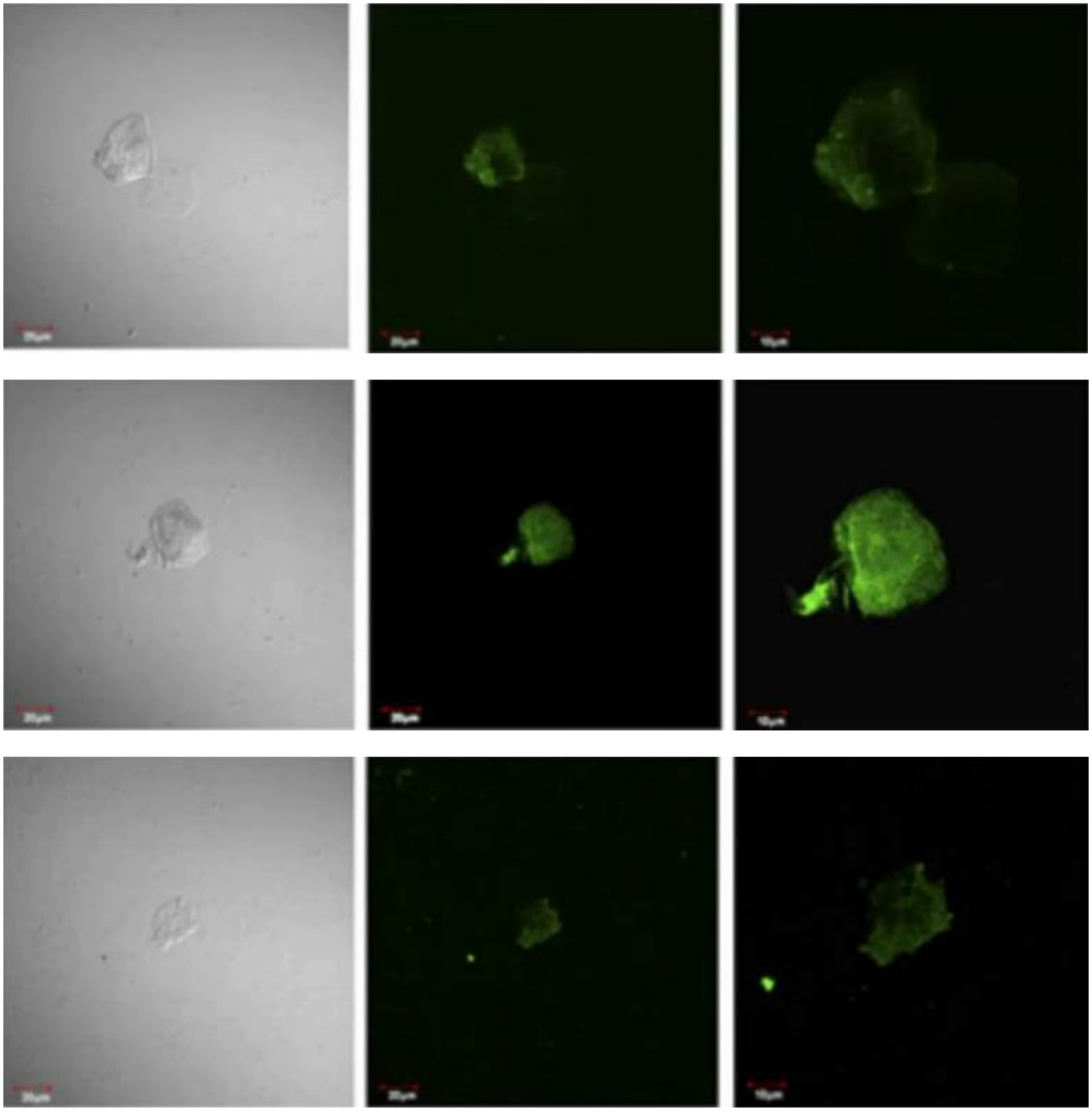


Figure 1: Autofluorescence, 1A-C) Sample A3 direct 1 D-F) Sample P2 direct. 1 G-1) Sample Si direct. Excitation wavelength 488 nm/ Emission wavelength 530 nm, 4000 magnification, 2X zoom. Confocal Laser Microscope FV1000 Oltympus-Japan.

ELECTRON MICROSCOPY

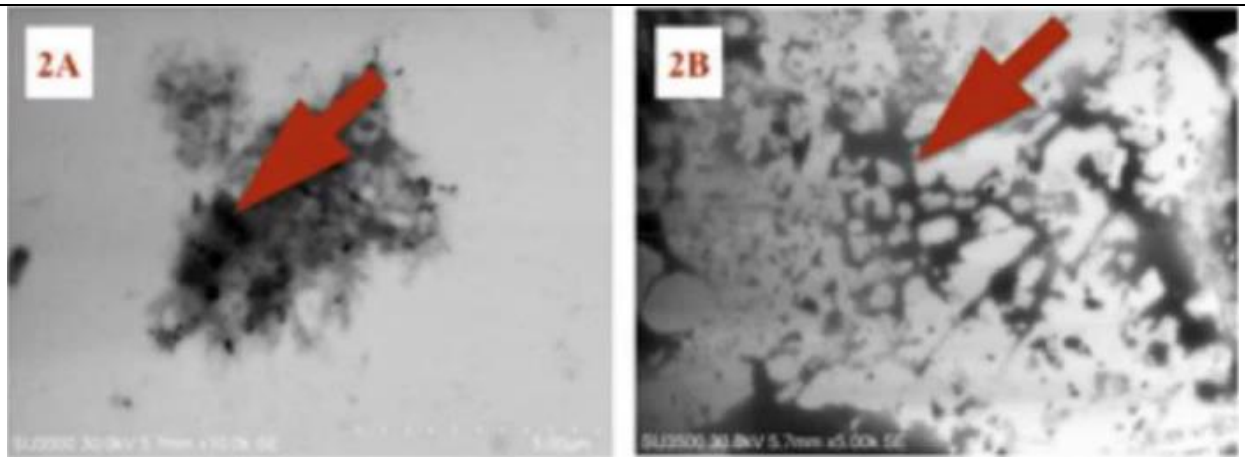


Figure 2: **Effect of sample extraction type.** 2A) STEM A1 10000 magnification. 2B) STEM A2. 5000 magnification. Samples with grid mount -Cu coated with carbon polymer, 1:50 dilution, SE detector, Scanning Electron Microscope SU3500 HITACHI-Japan.

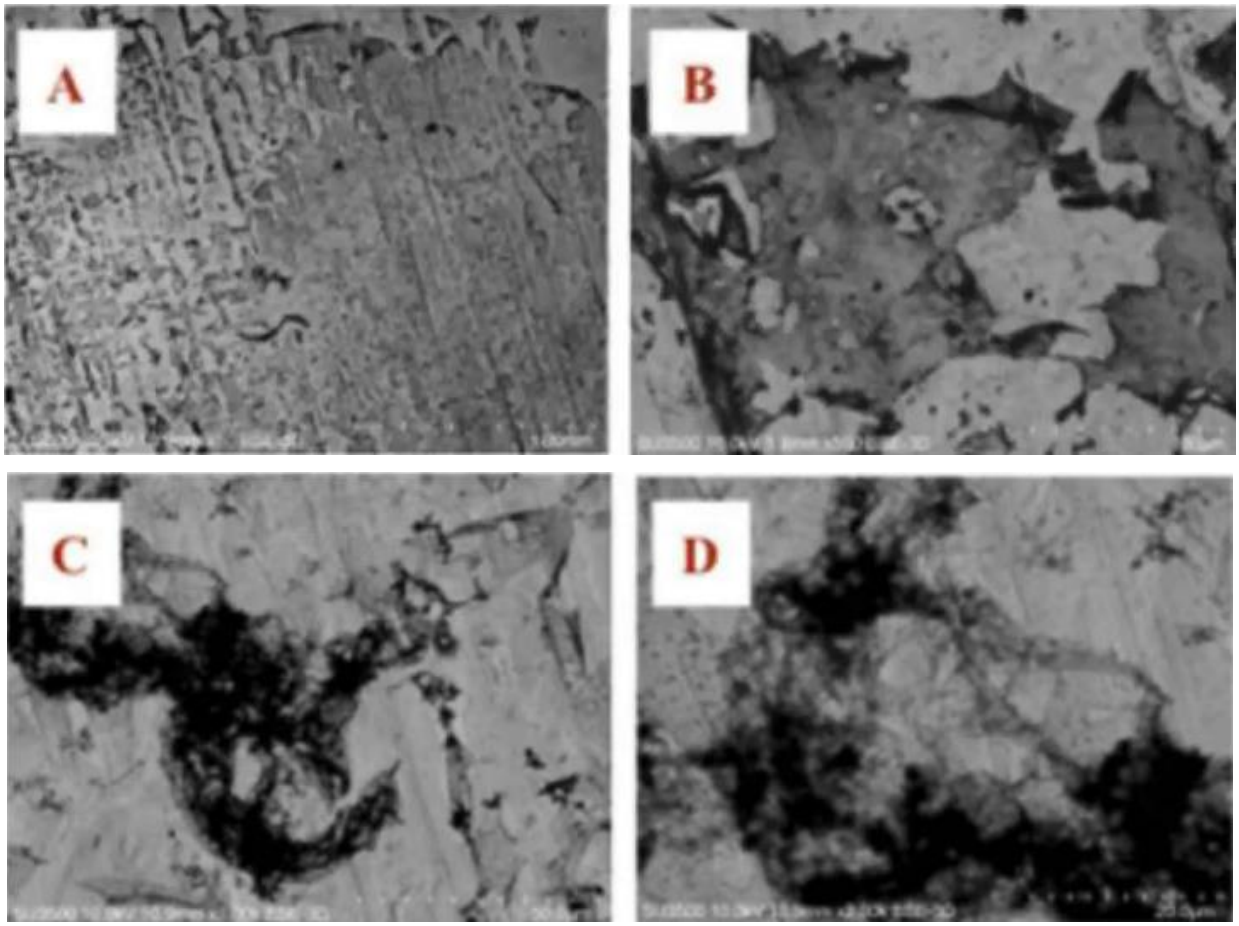


Figure 3: SEM-BSD (chemical contrast) Sample A3. detected BSD, Cu foil mount, 50-2000 magnification. dilution 1/50. Bamdo Electron Microscope SU3500 HITACHI-Japan.

ELECTRON MICROSCOPY

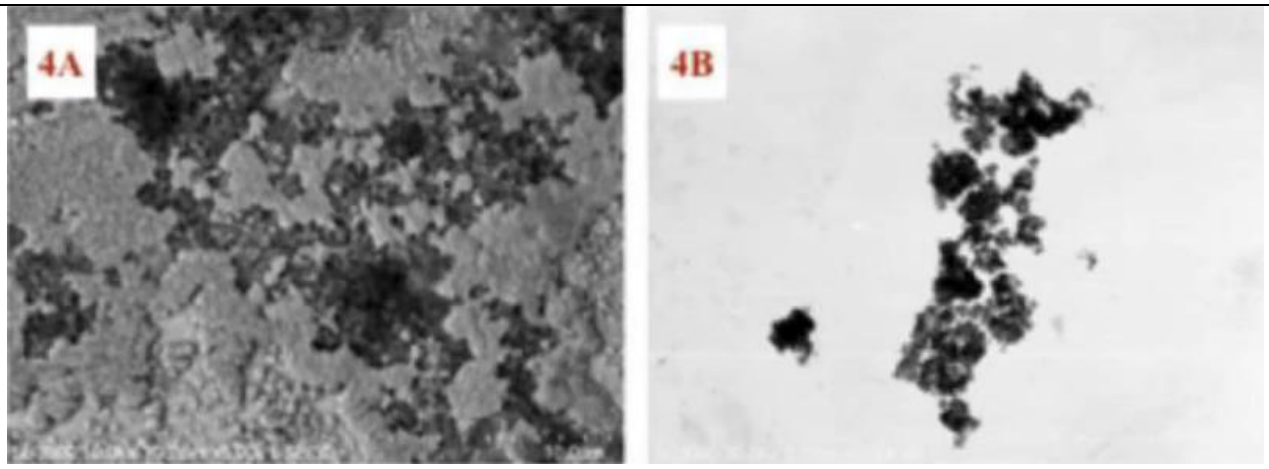


Figura 4. Comparative SEM and STEM visualization. 4A) SEM sample S1: Cu slide mount, dilution 1:30. BSD detector 5000x magnification. 4B) STEM sample S1: grid mount -Cu coated with carbon polymer. 1/50 dilution, SE detector. 5000 magnification. Scanning Electron Microscope SU3500 HITACHI-Japan.

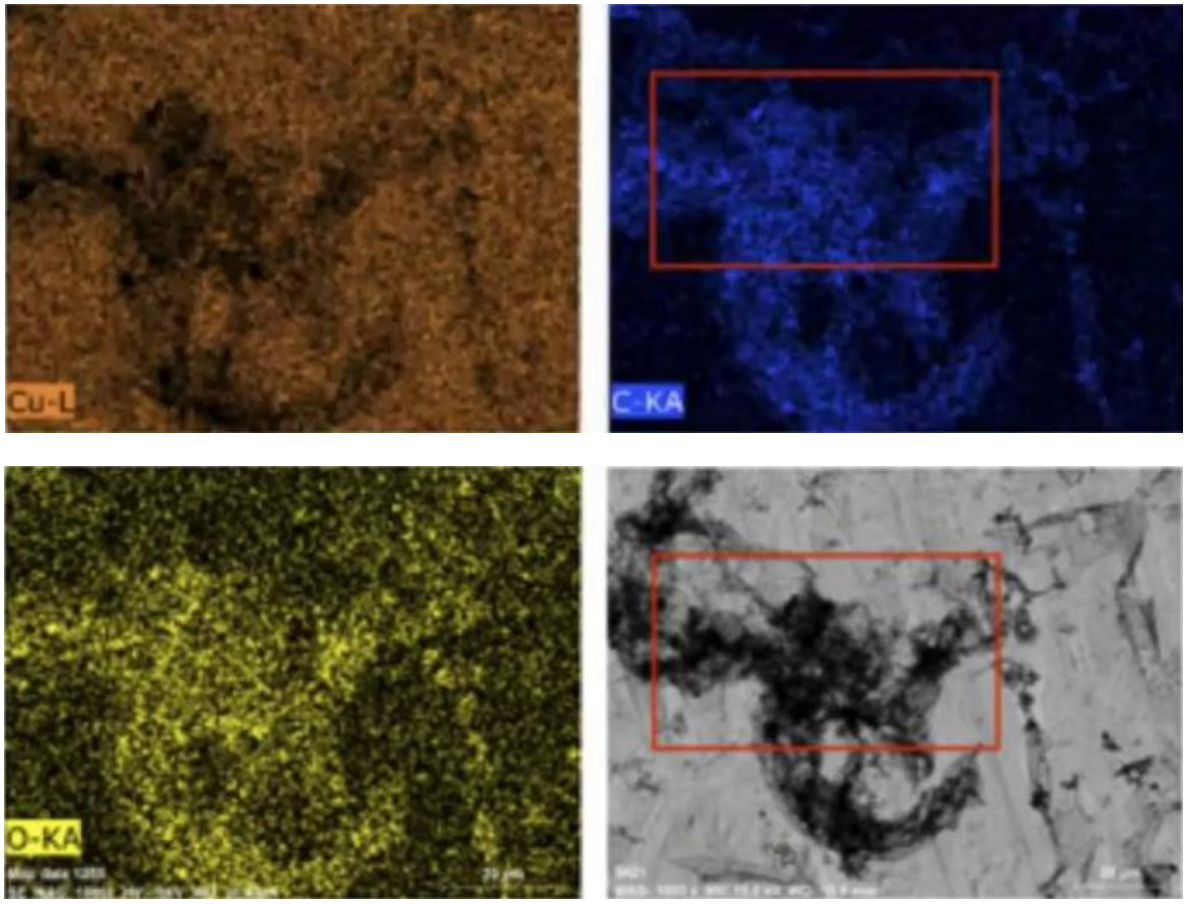


Figura 5. Elemental Distribution (Mapping). SEM-EDX A3. 5000 magnification. Samples with Cu mounting, dilution 1:50, BSE detector coupled to EDX, HITACHI-Japan SU3500 Scanning Electron Microscope. EDX Quantx: Bruker-Germany.

CONCLUDING OBSERVATIONS

The images of the samples obtained by Confocal Laser Microscopy (Figure 1) were analyzed directly, finding microstructures that present autofluorescence with greater intensity at 530 nm, characteristic of the presence of organic matter.

Standardization was performed for direct sample visualization to SEM in an aluminum sample holder. A random sample was observed, and it was observed that it was highly concentrated and reacted easily to the electron beam, altering the morphology.

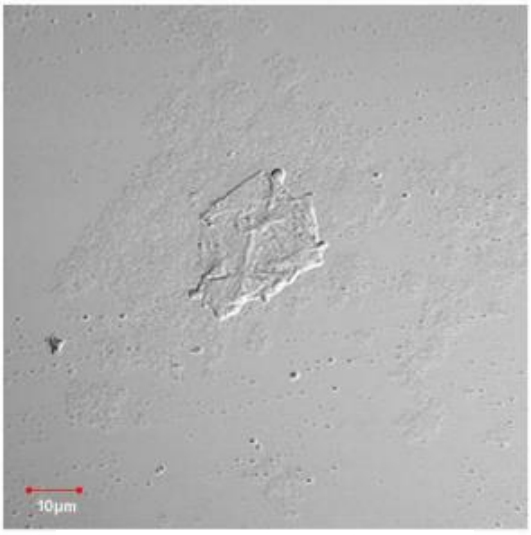
Dilution 1:50 was assayed, mounted for STEM on a copper grid coated with carbon polymer (Figure 2). Sample A1 and A2 show oily inclusions probably attributed to the extraction process and may correspond to trapped volatiles (Figure 2 A and B).

Dilution 1:50 was tested, samples were mounted for SEM on Cu stub (A3), and structures like sheets easily detachable from the stub were observed. When magnified, amorphous structures forming meshes were observed (figure 3).

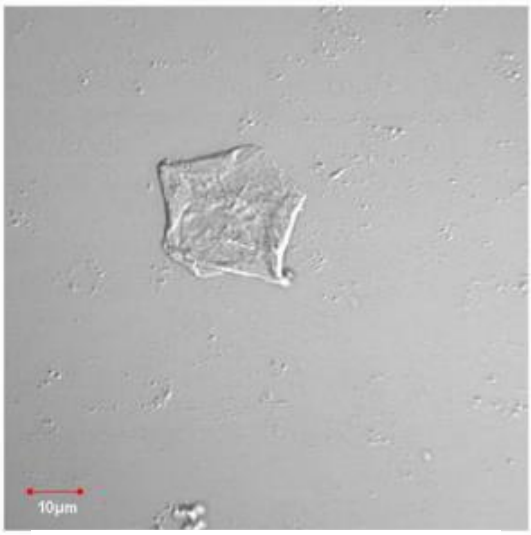
We proceeded to compare the images obtained both in SEM-Cu and STEM. Sample S1 diluted 1:50 for SEM-Cu shows amorphous structures forming meshes . There are still salts in the sample (Figure 4A). STEM visualization shows amorphous agglomerates of nanostructures (Figure 4B).

An elemental distribution mapping was performed on SEM-Cu mounted samples, diluted 1:50, found that the amorphous structures present correspond to carbon and oxygen. In some samples, there's presence of chlorine and sodium due to the presence of salts from the extraction processes.

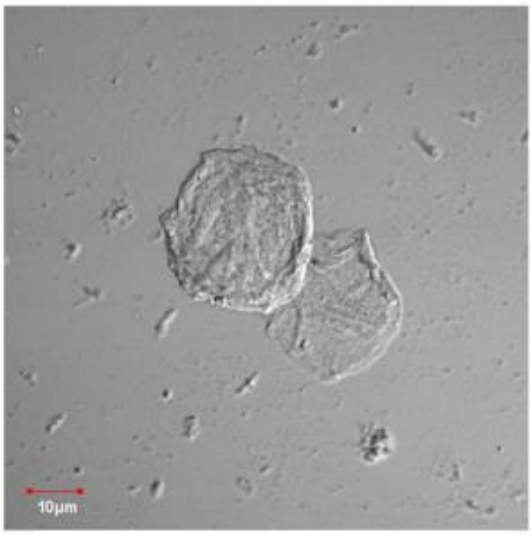
60X CONFOCAL IMAGES



SINOVAC VACCINE [CoronaVac]



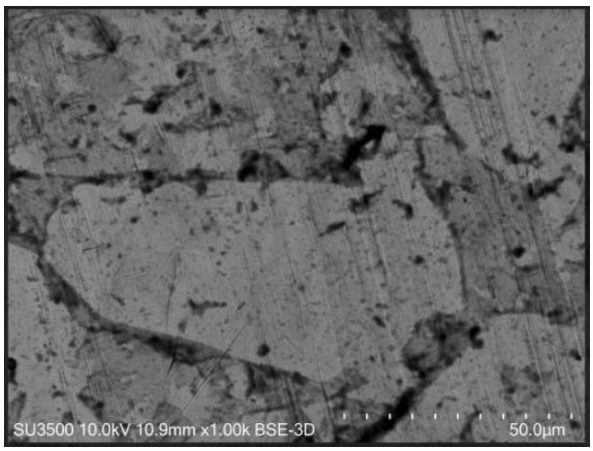
ASTRAZENECA VACCINE [ChAdOx1-S]



PFIZER VACCINE [COMIRNATY]

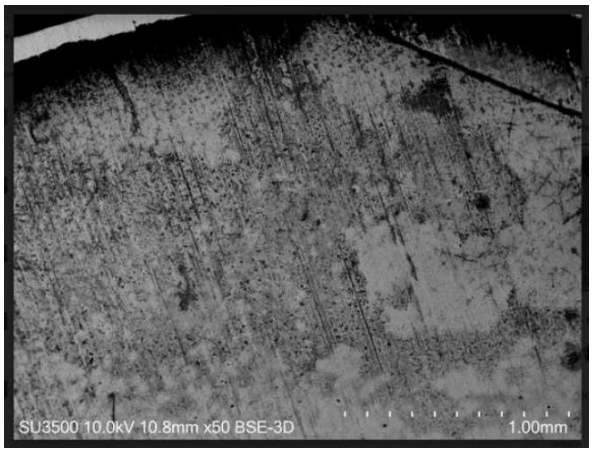
SCANNING ELECTRON MICROSCOPY IMAGES SEM

ASTRAZENECA



SINOVAC

PFIZER



IMPORTANT: This is an unofficial translation that **Orwell City** made of the summary for the general public of the AstraZeneca, Pfizer, and Sinovac vaccination vials analyses carried out in Chile. For more details and/or to request further information for scientific/research purposes, please refer to **Radio El Mirador del Gallo** and/or **La Quinta Columna**.