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TITLE:

Obtaining 3D Chemical Maps by Energy Filtered Transmission Electron Microscopy Tomography

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KEYWORDS:

3D chemical mapping, EFTEM tomography, analytical tomography, silica alumina, titania alumina, catalyst support

SUMMARY:

This paper describes a protocol to achieve 3D chemical maps combining energy filtered imaging and electron tomography. The chemical distribution of two catalyst supports formed by elements that are difficult to distinguish by other imaging techniques was studied. Each application consists of mapping overlapped chemical elements - respectively spaced-ionization edges.

ABSTRACT:

Energy filtered transmission electron microscopy tomography (EFTEM tomography) can provide three-dimensional (3D) chemical maps of materials at a nanometric scale. EFTEM tomography can separate chemical elements that are very difficult to distinguish using other imaging techniques. The experimental protocol described here shows how to create 3D chemical maps to understand the chemical distribution and morphology of a material. Sample preparation steps for data segmentation are presented. This protocol permits the 3D distribution analysis of chemical elements in a nanometric sample. However, it should be noted that currently, the 3D chemical maps can only be generated for samples that are not beam sensitive, since the recording of filtered images requires long exposure times to an intense electron beam. The protocol was applied to quantify the chemical distribution of the components of two different heterogeneous catalyst supports. In the first study, the chemical distribution of aluminum and titanium in titania-alumina supports was analyzed. The samples were prepared using the swing-

pH method. In the second, the chemical distribution of aluminum and silicon in silica-alumina supports that were prepared using the sol-powder and mechanical mixture methods was examined.

INTRODUCTION:

The properties of functional materials are dependent on their 3D parameters. To fully comprehend their properties and enhance their functions, it is important to analyze their morphology and chemical distribution in 3D. Electron tomography¹ (ET) is one of the best techniques to provide this information at the nanometer scale^{2,3}. It consists of rotating the sample over a large angular range and recording one image at each angular step. The obtained tilt series is used to reconstruct the volume of the sample by using mathematical algorithms based on the Radon transform^{4,5}. Selecting grey levels in the volume helps to model the sample in 3D and quantify 3D parameters like particle localization⁶ and size distribution⁷, pore position and size distribution⁸, *etc.*

In general, ET is performed with an electron microscope by tilting the sample to the maximum possible angle, preferably more than 70° in either direction. At each tilt angle, a projection of the sample is recorded forming an images tilt series. That tilt series is aligned and used to reconstruct the volume of the sample which will be segmented and quantified. Because the sample cannot be rotated from -90° to +90°, the reconstructed volume has an anisotropic resolution along the orthogonal axis⁹ due to the blind recording angle.

ET can be performed in different imaging modes. The bright field TEM mode (BF-TEM) is used to study amorphous materials, biological samples, polymers, or catalyst supports with complex shapes. The image analysis is based on the differentiation of the gray levels characterizing the density of the components¹⁰ (a dense component will be more dark than a lighter, *i.e.*, less dense component). High-angle annular dark field in scanning TEM mode (HAADF-STEM) is used to analyze crystalline samples. The signal provides chemical information as a function of the atomic number; a heavy component of the sample will appear brighter than a lighter one⁹. Other modes, like Energy Dispersive X-ray spectroscopy (EDX), which collects the X-ray emitted by the material¹¹, and energy filtered imaging mode (EFTEM)^{12,13}, are also capable of assessing the 3D chemical distribution within the sample.

In EFTEM imaging, the 2D chemical maps can be recorded using a TEM with an electron energy spectrometer. The spectrometer acts as a magnetic prism by dispersing the electrons as a function of their energy. An image is created by the electrons depending on the energy lost from interacting with a specific atom. If the same 2D chemical map is computed at different tilt angles, a tilt series of chemical projections is obtained, which can be used to reconstruct the 3D chemical volume.

Not all the materials can be analyzed by EFTEM tomography. The technique is reserved for samples with weak or disordered materials. Nevertheless, it can be used for analyzing light elements that are very difficult to differentiate when using other imaging techniques. In addition, to obtain reliable 2D chemical maps, the thickness of the material is required to be

less than the mean free path of the electrons through the material¹⁴. Under this condition, the probability of having a single electron interacting with a single atom is greatest. Two methods are used to calculate a 2D chemical map. The first one, and the most used is the “three-windows method”, where two filtered energy windows are recorded before the ionization edge of the element under analysis, and a third after the ionization edge¹³. The first two images are used to estimate the background, which is extrapolated using a power law at the position of the third window and subtracted from it. The obtained image is the projection of the 3D distribution of the analyzed chemical element in the sample volume. The second method is called the “jump-ratio”; it uses only two energy-filtered images, one before and one after the ionization edge. This method is qualitative, as the final image is calculated only by performing the ratio between those two images, and does not account for background energy variation.

By combining EFTEM with ET, the analytical tomography of the filtered energy can be obtained. EFTEM tomography and atom probe tomography (APT) are complementary techniques. As compared to APT, EFTEM tomography is a non-destructive characterization analysis that does not need complex sample preparation. It can be used to perform various characterizations on a unique nanoparticle. EFTEM tomography can analyze insulating materials, while APT needs at the very least laser assistance to measure them. APT runs at the atomic scale, while EFTEM tomography performs adequately with a lower resolution. EFTEM tomography is pertinent only for samples that resist beam degradation during the experiment. To record all the filtered images at all the tilted angles, the sample can be exposed to the electron beam for as long as 2 h. Moreover, to record a maximum chemical signal in the 2D maps, longer exposition durations at high beam intensity may be necessary. In such conditions, the beam sensitive samples suffer drastic morphological and chemical changes. Therefore, a precise measurement of the sample sensitivity to the electron beam must be established before the experiment. In addition, EFTEM tomography is the result of recording as many tomograms as necessary to determine the spatial location and nature of the chemical elements that are present in the sample. Nevertheless, EFTEM tomography can provide important information concerning the 3D chemical distribution for samples, such as catalyst supports, to give new insights for modeling their catalytic applications.

Today it is possible to use dedicated software that can select the energy interval, record filtered energy window images, and calculate the chemical maps at different tilt angles. They allow tilting the sample, tracking, focusing, and recording the filtered image in EFTEM mode. The 2D chemical maps can be calculated, and then the tilt series can be aligned, the chemical volume computed using iterative algorithms, and finally the series can be segmented and quantified¹⁵⁻¹⁶.

PROTOCOL:

1. Sample Preparation

1.1. Crush the sample in a mortar and disperse it in alcohol or distilled water; place a droplet of the sample on a microscopy grid and let it dry.

Note: Samples such as silica alumina or titania alumina can be a powder or an extruded material, and can be crushed and dispersed in a solution using ultrasound. In general, for ET analysis, it is important that the sample concentration on the grid is low, to avoid sample superposition and shadowing when tilting the grid at large angles. The 200-mesh microscopy grids that support a film of holey carbon or lacey carbon are recommended.

1.2. Using a pipette, put a droplet of a colloidal solution containing fiducial markers over the sample. Absorb any excess solution and let it dry.

Note: The fiducial markers are well-calibrated Au nanoparticles suspended in a solution. The fiducial markers can be also dispersed over the grid before adding the sample. As an example, if the sample is made from nanoparticles with similar sizes as the fiducial markers, deposit the fiducial markers over the grid in order to separate them well during the data segmentation and quantification. The fiducial markers are position references used when aligning the tilted images.

2. Recording of the Filtered Tilt Series Images

2.1. With the electron microscope, find an isolated sample in the center of the microscopy grid.

Note: In the electron microscope, the X axis is along the sample holder, the Y axis is perpendicular to the sample holder, and the Z axis is along the electron beam. To be able to tilt the sample at the maximum tilt angle, analyze a sample situated as close as possible to the X axis.

2.2. Once the sample is well-positioned, check the sample's chemical composition. Perform a chemical analysis using energy dispersive X-ray spectrometry (EDS), or electron energy loss spectrometry (EELS) by focusing the beam over the chosen sample, and record a spectrum. If the sample contains the chemical elements of interest, move away from it and run the next tests on a nearby representative sample.

2.3. Check the electron beam intensity over the sample, the width of the energy windows of the filtered images, and the exposition time of each filtered image. Find the best compromise for the sample between beam damage and the chemical signal recorded in the chemical projections^{12,13,17}.

Note: To record the maximum chemical signal in the filtered images, use the maximum beam intensity. However, an irradiation test must be performed before any analysis to check for any changes to the sample from the effect of the electron beam. To do this, calculate the electron dose during the recording of the tilt series. In addition, an ease test can be done by comparing an image before and an image after the experiment.

2.4. Use the EFTEM mode of the recording software to calculate a 2D chemical map using the

three-windows method, and check if a sufficient chemical signal is recorded.

Note: The software records three filtered images; the first two are used to estimate the background from the third image.

2.4.1. In the software, select the chemical element under investigation and its ionization edge. Set the width of the energy window and the exposure time. Record the images and then calculate the chemical map using a power law to extract the background. In a 32-bit environment with 512 x 512 pixels, the minimum signal is approximately 300–400 counts per pixel recorded in a chemical image.

2.5. Set the sample at the eucentric height and check the minimum tilt angle, *i.e.*, -70° or less, and maximum tilt angle, *i.e.*, $+70^\circ$ or more.

2.6. Bring the sample to be analyzed back into view and record an image (this will be the image before acquisition). Then record the tilted series of filtered images using appropriate software.

Note: The dedicated EFTEM tomography plugins can record several tilt series at the same time. This means that, at each tilt angle, several successive images can be recorded. The first image can be a filtered image centered on zero-loss and this image is a typical bright-field image. The pre-edge images and then the post-edge image of the first chemical element are followed by the pre-edge images and the post-edge image of the second chemical element. The succession of the chemical elements is given by their selected ionization edge.

2.6.1. In the recording EFTEM tilt series software, select the width of each energy window and its exposure time, and then the maximum and minimum tilt angle and the angular step of the tilt. To compromise between the number of images in the tilt series and the total exposure time of the sample to the electron beam, use a tilt step of 4° , *i.e.*, 51 images per tilt series between $\pm 71^\circ$; however, a smaller tilt step can be chosen if the sample does not degrade under the beam.

2.6.2. For each chemical element, record three tilt series of filtered images to calculate the chemical projection using the three windows method. To quantify the natural drift of the sample at each tilt angle while recording the filtered images (the sample can remain at certain tilt angles for more than 1 min), the first image can be a filtered image on zero loss signal so that the last recorded image will be an unfiltered image formed by all the electrons at all energies. Those two images can be used to calculate the thickness map of the sample. Therefore, for observing the chemical distribution of two elements at each tilt angle, record 7 filtered images (1 zero loss, 3 for the first chemical element, 3 for the second chemical element) and 1 unfiltered image (in total, 8 tilt series recorded).

3. Alignment and Reconstruction of the Tilt Series

3.1. Align the three filtered images corresponding to each chemical element for each tilt angle, and calculate the chemical map using a specialized EFTEMJ^{15,18} plugin of ImageJ. In the ImageJ

software, use the path **File | Open** and select the files corresponding to the filter images tilt series. Open all three filtered tilt series: two pre-edge and one post-edge.

3.1.1. Open the dedicated EFTEM/TJ plugin. Click on **+ | Image or Stack** and select the tilt series that are already open.

3.1.2. In the table that appears, fill the **Energy shift** for each tilt series, *i.e.*, the energy at which each tilt series was recorded. Also, fill the **Slit width**, *i.e.*, the energy windows. Fill the **“Exposure time”** of each filtered image. Check for all three tilt series **Mapping**. Click on **Next**.

3.1.3. Chose the first image as the reference image and then click **Apply**. Click on **Next**: a preview image of the proposed alignment appears. Visually verify that the 3 images recorded at the same tilt angle are well superposed (no shift between the images should be observed).

Note: This protocol was performed on the version 0.9 of the EFTEM/TJ plugin. At this moment, the filtered images recorded at the same tilt angle are aligned.

3.1.4. Select on the EFTEM/TJ window, the images corresponding to the **Background** and to the chemical **Edge**. Select the model of the signal extraction as **Power**, and then click on **Create map**. On ImageJ, select **File | Save** and find the path to save this last file.

Note: The tilt series of the chemical map is obtained. More information on how to use the plugin is available online.

3.1.5. Repeat step 3.1 for all the chemical tilt series.

3.2. Align the zero-loss tilt series by using a version of the Imod software¹⁹, released in 2009 to align the tilt series. The software allows the application of the calculated alignments on a tilt series to another tilt series.

Note: The alignment software writes on the disk files containing all the displacements applied to each image. The alignment procedure using Imod²⁰ is reviewed elsewhere and is not within the scope of this article.

3.3. Use the alignment calculated for the zero-loss tilt series, and apply it to the previously calculated chemical tilt series.

Note: In this software version, it is possible to change the zero-loss tilt series file with a chemical tilt series file by preserving the name of the file and applying the previous calculated displacements. Otherwise, for the software, the file will have the same name, the same number of images of the same size, but it will not contain zero loss images but chemical images.

3.4. Quantify the drift by cross correlating the first recorded image, *i.e.*, center on the zero loss, and the last one (the unfiltered image). The sample can spend several seconds at every tilt

angle while all the filtered images are recorded. During this time, the sample naturally drifts a small amount.

3.4.1. On ImageJ software, click on **File | Open** and select the zero loss aligned tilt series, then open the chemical maps aligned tilt series.

3.4.2. Click on **Edit | Color | Merge Channels**. Select the file corresponding to zero loss for the red color, the first chemical element for green, and the second chemical element for blue, in that order. Uncheck **Create Composite** and check **Keep Source Image**. A stack is created at each tilt angles for all the recorded images.

3.4.3. Click on **Plugins | Align RGB plans**²¹. Red is the reference image. Select green and using the arrows, overlap it over the red one. Then, select blue and overlap it over the red image. Click on next and repeat for all angles.

3.4.4. Click on **Edit | Color | Split Channels** and the RGB stack will be split in three stacks: red corresponding to zero loss, and green and blue corresponding to the chemical maps with the drift corrected. Click on **File | Save** to save the tilt series.

3.5. Click on **Plugins | Tomoj**^{22,23} to select the load angle form file. Because all the tilt series are already aligned, navigate directly to **Reconstruction**. Calculate the volumes of zero-loss, as well as the chemical volumes, using reconstruction algorithms like ART, SIRT, OS-ART, *etc.*

Note: It is recommended to use an iterative algorithm for the reconstruction of the chemical volumes. By using this software, it is possible to reconstruct the volumes using the GPU.

3.6. Once all volumes are computed, use the **Merge Channels** option to apply different colors to the obtained volumes and overlap them in a single volume, to obtain the 3D chemical map.

4. 3D Modeling and Quantification

4.1. Binarize the reconstructed ZL volume by selecting the corresponding gray level, which will be the volume obtained in white (in 8 bit, the intensity is 255) and black (in 8 bit, the intensity is 0). In ImageJ, use the “select threshold” option. Select all the pixels corresponding to the sample (in a BF image, the darker pixels correspond to the sample mass) and make a volume where the sample is white and the vacuum is black.

Note: The zero-loss volume provides morphological information of the analyzed sample, *i.e.*, the shape and the size of the sample.

4.2. Divide the binary volume of zero loss by 255, and obtain a volume where the intensities inside the sample are 1 and elsewhere are 0. This is the normalized volume.

4.3. Multiply the normalized volume by each of the calculated chemical volumes (step 4.1) to

obtain a volume where the intensities inside the sample correspond to the chemical information, and these intensities are 0 elsewhere.

Note: The chemical information is derived from the sample and therefore, all artifacts are excluded.

4.4. In ImageJ, calculate the histogram of the chemical volume and import the values of the histogram in a tabulate software.

4.4.1. In the tabulate software, delete the line with an intensity heist count of 0 (this line corresponds to the vacuum).

4.4.2. In a new column, calculate the proportion of each intensity in the volume. Divide the count of each intensity by the sum of all counts, and multiply it by 100.

4.4.3. In a new column, calculate the proportion corresponding to the intensity of the volume total by incrementally adding the current proportion of the counts, calculated previously with the previous proportion.

Note: In the chemical volumes, the high intensities correspond to the chemical information. However, the intensities are low and the noise in the volume high. The threshold is created by selecting the highest intensities.

4.4.4. Knowing the relative concentration of the chemical element in the sample calculated from the EELS spectrum, select the heist intensities. Start from 255 (if existing) and decrease intensity corresponding to the chemical element concentration.

4.4.5. To find the lowest intensity corresponding to the chemical concentration, navigate to the column where the proportion of corresponding intensity was calculated in the total volume, the 100% corresponds normally to the intensity of 255. From 100%, extract the calculated relative proportion of the chemical element (from the EELS spectrum): the result will correspond to the minimum intensity in the threshold. In this way, chemical binarized volumes are obtained, where the voxels correspond to the chemical element with the intensity of 255 and all the rest are 0. Repeat the procedure for the second element and obtain two chemical binarized volumes.

4.5. Overlap the binarized chemical volumes using the **Merge Channels** option and assign a different color to each element volume to make an RGB volume.

Note: By overlapping the two binarized chemical volumes, voxels that are superimposed, which belong to both chemical elements (commune voxels) and voxels that do not belong to any of the chemical elements (free voxels) are highlighted. For example, the binarized chemical volumes of red and green create commune voxels that are yellow.

4.6. Transform the RGB volume in 8 bits volume; the colors will have different grey intensities. Using the threshold option, select the voxels that belong to both chemical species (yellow in the RGB volume). Then, using the same threshold option, select the voxels that do not belong to any chemical element (they have lower intensities than the voxels selected earlier). Do not select the vacuum with intensity 0.

4.7. Normalize the volumes of the commune voxels and the volume of the free voxels. Multiply the volumes of the free voxels by the chemical volumes, then subtract the other chemical volume.

Note: This calculates the chemical element in each voxel that has the highest intensity.

4.8. Add the volumes of the voxels that belong and the voxels that do not belong to the volume of the chemical element that has the highest intensity in those voxels.

Note: In this way, each commune voxel or free voxel is differentiated and allocated to the volume of the chemical element that has the highest intensity in that voxel. This can be performed by the "Image calculator" option:

$$\begin{aligned}
 & \text{Voxels commune of element 1} \\
 & = \left(\frac{\text{Total commune voxels}}{255} * \text{chemical volume 1} \right) \\
 & - \left(\frac{\text{Total commune voxels}}{255} * \text{chemical volume 2} \right)
 \end{aligned}$$

$$\begin{aligned}
 & \text{Voxels commune of element 2} \\
 & = \left(\frac{\text{Total commune voxels}}{255} * \text{chemical volume 2} \right) \\
 & - \left(\frac{\text{Total commune voxels}}{255} * \text{chemical volume 1} \right)
 \end{aligned}$$

$$\begin{aligned}
 & \text{Free voxels of element 1} \\
 & = \left(\frac{\text{Total free voxels}}{255} * \text{chemical volume 1} \right) \\
 & - \left(\frac{\text{Total free voxels}}{255} * \text{chemical volume 2} \right)
 \end{aligned}$$

$$\begin{aligned}
 & \text{Free voxels of element 2} \\
 &= \left(\frac{\text{Total free voxels}}{255} * \text{chemical volume 2} \right) \\
 &- \left(\frac{\text{Total free voxels}}{255} * \text{chemical volume 1} \right)
 \end{aligned}$$

$$\begin{aligned}
 & \text{Total voxels of element 1} \\
 &= \text{Element 1 binarized} - \text{Voxels commune of element 2} \\
 &+ \text{Free voxels of element 1}
 \end{aligned}$$

$$\begin{aligned}
 & \text{Total voxels of element 2} \\
 &= \text{Element 2 binarized} - \text{Voxels commune of element 1} \\
 &+ \text{Free voxels of element 2}
 \end{aligned}$$

4.9. To quantify the number of voxels that form the sample, import the segmented volumes into specialized surface rendering software, such as a 3D Slicer. Then, multiply the volume of a voxel in nm³ to obtain the volume of the sample in 3D.

4.10. Using the **Find Edges** option, quantify the voxels forming the surface of the sample, and multiply with the voxel area in nm² to obtain the surface of the sample.

4.11. Calculate the specific surface area by dividing the surface of the sample by the mass of the sample.

Note: The mass of the sample can be estimated by using the theoretical density of the sample. In general, the specific surface area calculated by ET is 10 less than the specific surface calculated by dedicated methods such as N₂ adsorption desorption.

4.12. To calculate the pore size distribution, use the binarized volume of the zero loss (the BF volume). The binary volume of the BF reconstruction is dilated using the 3D Toolkit/Morphological dilate 3D plugin until all the pores are covered and then eroded using the 3D Toolkit/Morphological erode 3D as many times as dilated. Then, the obtained volume is multiplied by the inverted volume of the binarized BF volume, to result in the pores distribution volume that can be visualized using a dedicated surface rendering software.

REPRESENTATIVE RESULTS:

An example of the application of this protocol is shown in reference¹³. EFTEM tomography was used for analyzing titania alumina catalyst supports. To enhance the catalytic activity of the

active phase of MoS₂ nanoparticles, in applications like hydrodesulfurization (HDS), it is important that titania is preponderant at the support surface, and in contact with the active phase. It is known that titania has a smaller specific surface than alumina. The aim of the study is to construct titania supported by alumina (and thus, create an enhanced specific surface), and then to test it as a catalyst support. Here, the EFTEM tomography is used to analyze of titania-alumina heterogeneous catalyst support prepared by the swing pH method. In this study, three samples of different titania concentrations are analyzed. Sample 1 is composed of 50% alumina and 50% titania, Sample 2 is composed of 70% alumina and 30% titania, and Sample 3 is composed of 90% alumina and 10% titania. In **Erreur ! Source du renvoi introuvable.a-1c**, cross sections of the chemical maps parallel to the XY plane are shown. Green represents the spatial chemical distribution of titania, red represents the distribution of alumina, and blue represents the vacuum. The chemical volumes are calculated from the tilt series with the three-windows method. The filtered energy windows were as follows: 10 eV wide, centered at 59, 70, and 81 eV, having an exposition time of 3 s for the Al L₂₃ edge situated at 73 eV and 30 eV wide, centered at 415, 445, and 482 eV, having a recording time of 15 s for the Ti L₂₃ edge situated at 455 eV. The position and the width, as well as the exposure time of the filtered images were chosen in order to obtain a chemical map with a detectable chemical signal. The tilt series were recorded between -71° and +71° with an increment of 4° in the Saxton's mode²⁴ in about 119 min.

It was found that titania at a high concentration is forming clusters that are embedded in the alumina. The models are displayed in **Erreur ! Source du renvoi introuvable.d** (Sample 1), **1e** (Sample 2), and **1f** (Sample 3). In the models, the titania is displayed in blue and the alumina is displayed in transparent red. These models were quantified using the chemical distribution of titania and alumina on the surface of the samples. It was found that independent of the proportion of titania and alumina in the sample, the surface of the sample is covered with titania in a proportion of 30%. However, the specific surface of the sample is increasing, while the titania proportion is decreasing to reach the specific surface of alumina. For Sample 3 containing only 10% titania, a layer of about 10 nm thick is formed on the surface of the sample. Also, the chemical map is formed by overlapping three volumes: silica in red, titania in green, and zero loss in blue. The mixture between red and green are yellow voxels. The yellow voxels are attributed in the model to the element having the highest intensity in that voxel. This is a limitation in the spatial resolution of 3D chemical maps, which is directly related to the anisotropic resolution in ET and the resolution of the 2D chemical maps provided by EFTEM. The analysis is correlated with other analytical techniques such as X-ray fluorescence, X-ray photoelectron spectroscopy, and N₂ porosimetry. It was concluded that the difference between the specific surfaces could play a role in the catalytic applications.

As a second example, the study detailed in ¹⁷ is shown. In this study, we analyzed a series of silica alumina catalyst supports. These catalyst supports have an acidity provided by the mixture between the alumina and silica, forming an aluminosilicate. The goal of the study was to quantify the mixture between the two components. The experimental challenge lied in the fact that the L₂₃ edges of Al and Si are very close, at 73 eV and 99 eV respectively, and the ionization edge of Al overlaps with the ionization edge of Si. Under these conditions, the three-window

method is less accurate for extracting the chemical signal. In order to differentiate the two signals of Al and Si, the “R-ratio” method was developed, detailed in reference ¹². The filtered images tilt series were recorded by tilting the sample from -71° to $+71^\circ$ with an increment step of 4° in the Saxton’s mode in about 83–104 min. Three filtered images were recorded to isolate the signal of the L_{23} ionization edge of Si. The images were centered at 59 eV, 70 eV, and 81 eV, were 10 eV wide, and exposed for 5 s. For the signal corresponding to the L_{23} edge of Al, only two filtered images were recorded, centered at 99 eV and 110 eV, 10 eV wide, and exposed for 12 s.

In this study, we analyzed a series of four samples of Al and Si prepared by different methods. Erreur ! Source du renvoi introuvable.**a** is a cross section of the chemical map parallel to the XY plane and the model of the sample prepared by the sol-powder method. This sample was thermally treated under steam, yielding the second sample, whose chemical map and model are displayed in Erreur ! Source du renvoi introuvable.**b**. Erreur ! Source du renvoi introuvable.**c** shows the chemical maps of the sample prepared by mechanical mixture. From this sample, after a thermal treatment under hot steam, we obtained the fourth sample, shown in Erreur ! Source du renvoi introuvable.**d**. The chemical maps and models for alumina are red and for silica are green, while the enhanced blue represents the boundary at the surface between the silica and alumina. The acid catalytic activity is given by the mixture between alumina and silica on the surface of the sample. It is found that independent of the preparation method, the silica covers only 30% of the sample surface. After the thermal treatment, the chemical distribution is more homogeneous, and the surface is covered with 50% silica and 50% alumina. The sol-powder method provides samples with a high homogeneity between the components compared with the mechanical mixture. Small domains of silica embedded in alumina are present in the sample. For the sample prepared by mechanical mixture, silica forms the core of the sample and alumina is present as a shell. As a general characteristic of both samples that are not thermally treated, the silica is in the center, and the alumina is on the surface.

The acid site density provided by the aluminosilicate phase formed by the intimate mixture between silica and alumina, creating Brønsted acid sites at its surface, is measured in arbitrary units (a.u.)/ m^2 by CO adsorption. The quantization of the boundary between silica and alumina was performed in m/g or m/m^2 , which are known physical units. Of course, the interface between silica and alumina could be thicker, but the spatial resolution reached did not allow the calculation of an exact value of the corresponding width. However, this study opens the way towards a deeper understanding of the interface between silica and alumina.

FIGURE LEGENDS:

Figure 1: Cross sections and reconstructed models of the titania and alumina samples. Cross sections through the chemical map parallel to the XY plane, where the chemical distributions are shown for titania (in green), alumina (in red), and the vacuum (in blue): **(a)** Sample 1, 50% alumina and 50% titania, **(b)** Sample 2, 70% alumina and 30% titania, and **(c)** Sample 3, 90% alumina and 10% titania. **(d)** **(e)** and **(f)** display the models of Sample 1, Sample 2, and Sample 3, respectively, with titania in blue and alumina in transparent red. In Sample 1 and Sample 2, the alumina embeds the titania. In Sample 3, a thin layer of 10 nm of titania is formed on the

surface of the sample. This figure has been modified from Roiban *et al.*¹³

Figure 2: Cross sections and reconstructed models of the silica and alumina samples. On the left are cross sections parallel to the XY plane from the chemical volumes, on the right are the reconstructed models. Alumina is shown in red, silica in green, and the boundary between the surfaces of the silica and alumina are indicated in blue. In those models, the boundary is artificially dilated by a 4-voxel sphere to make it visible. **(a)** The sample prepared by the sol-powder method, **(b)** the sample prepared by the sol-powder method and thermally treated, **(c)** the sample prepared by the mechanical mixture method, and **(d)** the sample prepared by the mechanical mixture method and thermally treated. This figure has been modified from Roiban *et al.*¹⁷

DISCUSSION:

The aim of this paper is to describe how to obtain 3D chemical maps using EFTEM tomography. This protocol is completely original and was developed by the authors.

EFTEM tomography as described here has several drawbacks: (i) Only samples that are electron beam resistant can be analyzed, due to the long exposure time needed for obtaining filtered images. (ii) EFTEM tomography is sensitive to the diffraction contrast. (iii) Many of the alignments were performed manually. To obtain the 3D chemical map, the zero-loss volume and the chemical volumes need to be in a single system of coordinates. This necessitates that all the tilt series be aligned perfectly in the same coordinate system. This represents a long work period of at least two weeks per sample. Despite being time consuming, this protocol allows the computation of 3D chemical maps at a nanometric resolution. In addition, combined with other spectroscopic and analytic techniques such as, X-ray fluorescence, X-ray photoelectron spectroscopy, FTIR spectroscopy, or magic-angle-spinning (MAS) NMR spectroscopy, a full description of a functional material can be created.

The electron beam intensity is generally controlled by condensing the electron beam. The width of the energy window through which the filtered images are recorded, and their exposure time will influence the chemical signal intensity recorded in each chemical projection, which will be used as tilted projections to reconstruct the chemical volume. The exposure time of the filtered images will influence the total exposure time of the sample under an intense electron beam during the recording of the tilt series. If a sample remains for too much time under the beam, it can suffer drastic modifications. The width of the energy windows influences the background approximation using a power law to extract the chemical signal from the post-edge filtered image using the three-windows method.

Since it is a challenging technique, and time consuming, EFTEM tomography is not practical for wide scale implementation. However, new technical improvements such as the development of more sensitive spectrometers²⁵ and fast recording cameras^{26,27} (the cameras references list is a partial list) will reduce the total record time of the tilt series and will enhance the energetic resolution of the chemical maps. As mentioned before, many of the alignments are manual, from signal extraction and computation of the chemical projections to the alignment of all

projections on the same reference. The development of automatic procedures will create a more general use of EFTEM tomography in routine analysis.

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The authors have nothing to disclose.

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