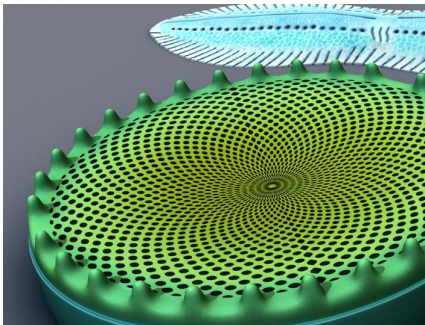




## METAL COATING OF BIOLOGICAL SAMPLES FOR ENHANCED SEM IMAGING: DIATOMS

### WHAT ARE DIATOMS?



Diatoms are a major group of microalgae, found in oceans, waterways and soils of the world. Living diatoms generate about 20 to 50 percent of the oxygen produced on the planet each year, take in over 6.7 billion metric tons of silicon each year from the waters in which they live, and constitute nearly half of the organic material found in the oceans.

Diatoms are unicellular. They occur either as solitary cells or in colonies, which can take the shape of ribbons, fans, zigzags, or stars. Individual cells range in size from 2 to 200 micrometres. A unique feature of diatom anatomy is that they are surrounded by a cell wall made of silica. Similar to plants, diatoms convert light energy to chemical energy by photosynthesis.

### WHAT ARE DIATOMS USED FOR?



Diatomaceous earth (diatomite) is a collection of diatom shells found in the earth's crust. They are soft, silica-containing sedimentary rocks which are easily crumbled into a fine powder and typically have a particle size of 10 to 200  $\mu\text{m}$ . Diatomaceous earth is used for a variety of purposes including for water filtration, as a mild abrasive, in cat litter, and as a dynamite stabiliser.

### WHY IS SEM IMAGING USED TO STUDY DIATOMS?

Diatoms are used to help determine the origin of materials containing them. One important field of use is forensics. A typical application is to differentiate a death by submersion from a post-mortem immersion of a body in water. Because the composition of diatoms is specific for a specific location, a sample of diatoms can point to a specific site.

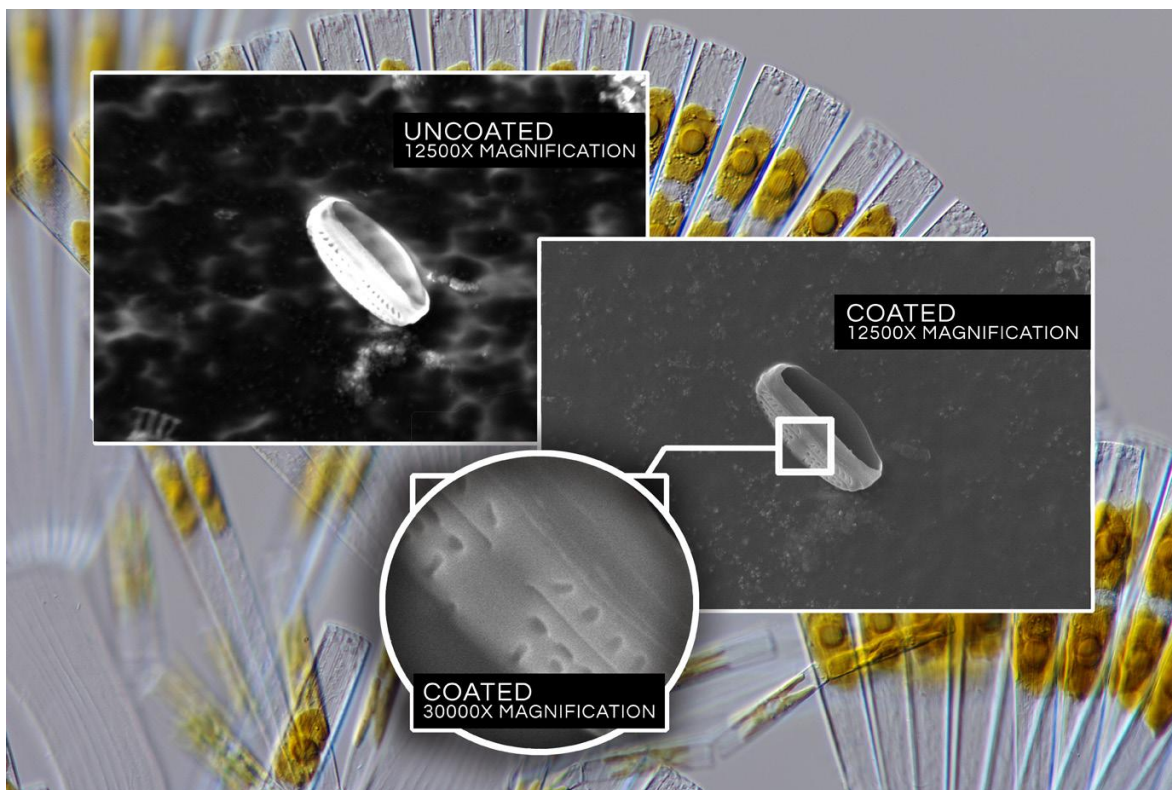
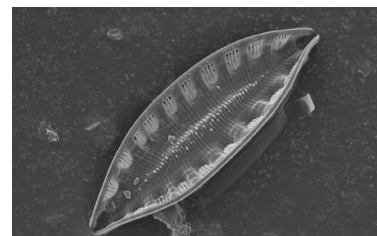
Diatoms are also used to monitor past and present environmental conditions, and are commonly used in studies of water quality.

The deposition of silica by diatoms may also prove to be of utility to nanotechnology. With an appropriate selection procedure, diatoms of particular shapes and sizes might be used to mass-produce nanoscale components. For all these applications SEM imaging is used to determine the shape, size and composition of a diatom sample.

## WHAT IS SAMPLE CHARGING?

What is sample charging, what are the effects of sample charging on SEM imaging of diatoms, and what are the positive effects of metal sputter coating?

SEM images are generated by scanning an electron beam across the sample. This effectively adds electrons to the sample. Sample charging occurs when samples are bad electrical conductors which means there is no conducting path for electrons to flow from the sample surface towards the sample holder. Sample charging causes all kinds of problems such as drift, blur, and low contrast. In other words, blurry and false images. By applying a very thin electrically conducting layer of metal such as gold or platinum (a process known as metal coating or sputter coating) onto the surface topography of the specimen, the electrons can flow from the sample surface towards the sample holder and sample charging is prevented. Other positive effects from sputter coating a sample are an improved secondary electron emission, a reduced beam penetration with improved edge resolution and a better protection of electron beam sensitive samples. The top image on the right shows the moderate charging effect of an uncoated diatom sample at 10 000X magnification. The bottom image is the same sample but coated with a 7 nm gold layer.



## IMAGING AND COATING CONDITIONS

SEM images were recorded with a Thermo Phenom XL desktop electron microscope using the SED detector in high vacuum mode (0.1 Pa) at 5kV. A 7nm gold coating was applied to the “coated” sample using the LUXOR Au metal coater.



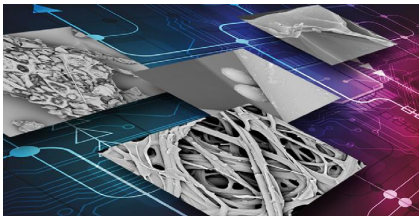
## SPUTTER COATING WITH THE LUXOR METAL COATER

LUXOR metal coaters are used extensively in SEM and TEM labs worldwide where image quality and high resolution imaging are of the utmost importance. Metal sputter coating not only prevents sample charging, but also provides improved edge resolution and a better protection of electron beam sensitive samples. Even at relatively low magnifications sample coating offers additional security in a high throughput environment with multiple operators having to provide high quality images in a routine analysis environment on a large variety of samples.



### A<sup>2</sup> TECHNOLOGY

LUXOR's unique A<sup>2</sup> Technology generates a metal plasma and applies it in a controlled and accurate manner, resulting in an extremely uniform, thin and homogeneous metal layer. The unique way this process is controlled and adjusted is what distinguishes the LUXOR metal coaters from other commercially available instruments. For the SEM operator this means more homogeneous metal coatings, resulting in high resolution and high contrast images and a worry-free coating process without any manual intervention.



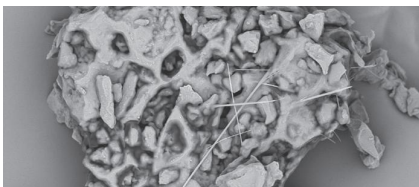
### UPSIDE DOWN DESIGN

In the LUXOR metal coaters, the samples are mounted upside down. While this might seem a little controversial at first sight, it is actually a consequence of our 'form follows function' approach. In fact, the upside down architecture brings many advantages. First, all high voltage and high current wires are safely hidden within the instrument. This obviously greatly reduces the risk of electric hazards. Next, the sample loading station is easily accessible and allows to apply or remove the samples without the need for special tongs or tweezers. This doesn't just make everyday use easier, but also speeds up productivity. The upside design also makes sure that loose particles will be removed during the coating process. This way, your SEM is optimally protected.



### FULL AUTOMATION

The coating process is fully automated. As soon as your samples are loaded into the preparation station, you only have to choose the desired coating thickness and push the start button. Thanks to this user friendly process, the chance of human errors is significantly reduced. Furthermore, this means that untrained operators and lab personnel can operate the device.



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