# A Novel Technique of Hair Removal to Examine the Cuticle of Arthropods

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The highly structured arthropod cuticle is often adorned with dense hairs that conceal the underlying landscape. Thus, to examine the structures and patterns on the cuticle the overlying hairs must be removed with minimal damage to the cuticle itself. Different methods can be employed to eliminate hairs but the difficulty of this task is compounded by the small size and fragility of the exoskeleton to which the hairs are attached. If the hairs are removed using razor blades or scissors, not only is the debris from the cut hairs and the stubble from the remainder of the shaft left behind, but the cuticle is often broken in the course of gripping the exoskeleton. We developed a unique technique that cleanly removes hairs without leaving debris or damaging the structure. This technique has been successfully used to examine the cuticular surfaces of arthropods of varying sizes including spiders, bees, beetles and moths.

## Methods

The sample to be examined is cut to a size that encompasses an area larger than the area of interest. A large drop of colloidal graphite is placed on an aluminum stub and spread to mirror the shape of the specimen. The graphite should be applied liberally so that a minimal amount of pressure is needed when placing the specimen on the stub. The specimen is then placed on the bed of colloidal graphite, with the area of interest facing down, into the liquid graphite. Care must be taken to lightly place the specimen on the graphite and allow natural capillary action of the hairs to draw up the liquid rather than using force to depress it into the graphite. Too much pressure can cause the specimen to sink into the colloidal graphite, causing the cuticle to be covered with the opaque graphite, rendering the specimen useless. Often, the specimen will draw up too much of the colloidal graphite, which will then begin to pool in the middle, making the specimen appear to be sitting on a mound rather then on a flat surface. When this occurs, a toothpick can be used to break the surface tension of the outer layer of the pooling graphite. Once the drying outer layer of graphite is penetrated, a pool of liquid flows from the mound, and the specimen will return to its original flat orientation. The colloidal graphite must uniformly adhere to the specimen because it keeps the specimen from prematurely breaking free when separating the hairs from the cuticle.

After drying the specimen for a minute, a second, small amount of graphite is applied to encircle the specimen to ensure a firm bond. If the specimen is not flat, multiple applications of colloidal graphite can be used to build a connection between the stub and the specimen. This occurs frequently with specimens because often the area of interest is curved and one part is touching the colloidal graphite while another area is not. It is imperative to have as much of the specimen touching the colloidal graphite as possible and as flat as possible to allow a firm bond once dried. This stage of preparation directly impacts how easily the cuticle can be separated as well as the quality of the image. The goal is to have the hairs on the surface of interest with as much contact to the colloidal graphite as possible without damaging the cuticle.

The specimen should be dried for a minimum of 24 hours. Because only the part adhered to graphite is used for examination, the excess portions of specimen can be trimmed using a razor blade. As an example, if the area of interest is the ventral side of an arthropod leg, then one would cut along the medial/lateral line using a razor blade to free the entire dorsal side of the leg. Once freed of the excess cuticle, the stub is oriented so that a razor blade, parallel to the face of the stub, can be used to carefully separate the cuticle from the hairs that are now firmly embedded in the colloidal graphite. If the graphite was properly applied to the specimen, the blade should easily separate the hairs from the cuticle. The blade is carefully moved forward until the area of interest is separated from its overlying hairs. Once the specimen is freed, it can be reaffixed to the stub, only this time with the area of interest facing up. The procedure for affixing the sample to a new stub requires a similar amount of pressure needed to initially fix the specimen hairs to the stub. Using a small amount of colloidal graphite, the cuticle is placed lightly on the stub. If too much pressure is used, the cuticle will sink into the graphite and become unusable.

If the graphite does not withstand the stress of using the razor blade to separate the specimen and breaks free from the stub, the razor blade can be used to carefully flake off the graphite. This will remove many of the hairs, but there is often more debris littered on the surface. It is also very difficult to hold the cuticle down with enough pressure to flake off the hairs without damaging the exoskeleton. This should only be done when there are no other options.

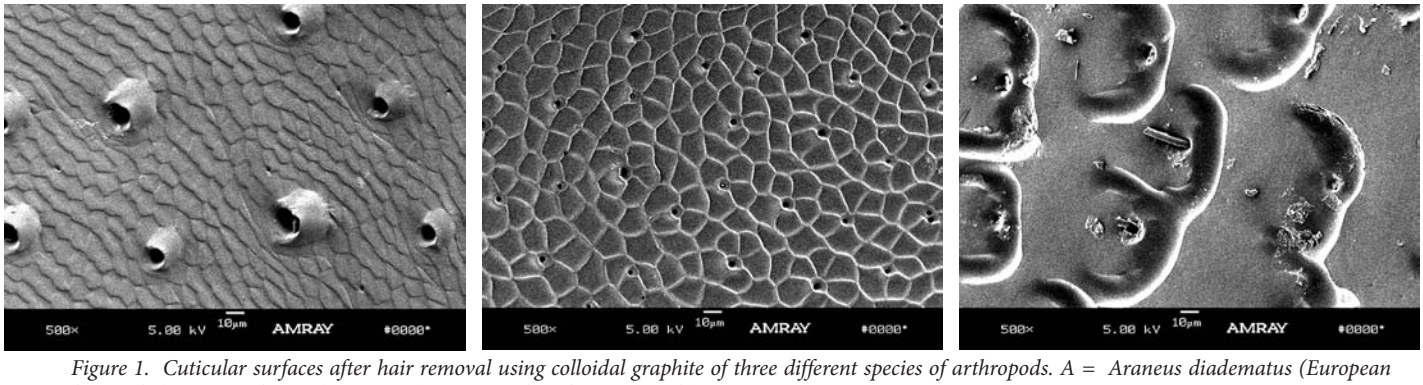


*Figure 1. Cuticular surfaces after hair removal using colloidal graphite of three different species of arthropods. A = Araneus diadematus (European Garden Spider); B = Apidae sp. (Honey Bee); C = Scarabaeidae sp. (Beetle).*

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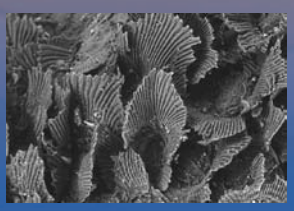
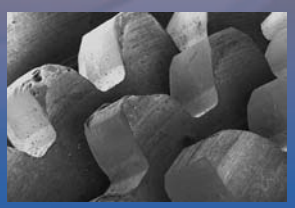
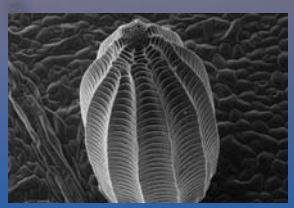



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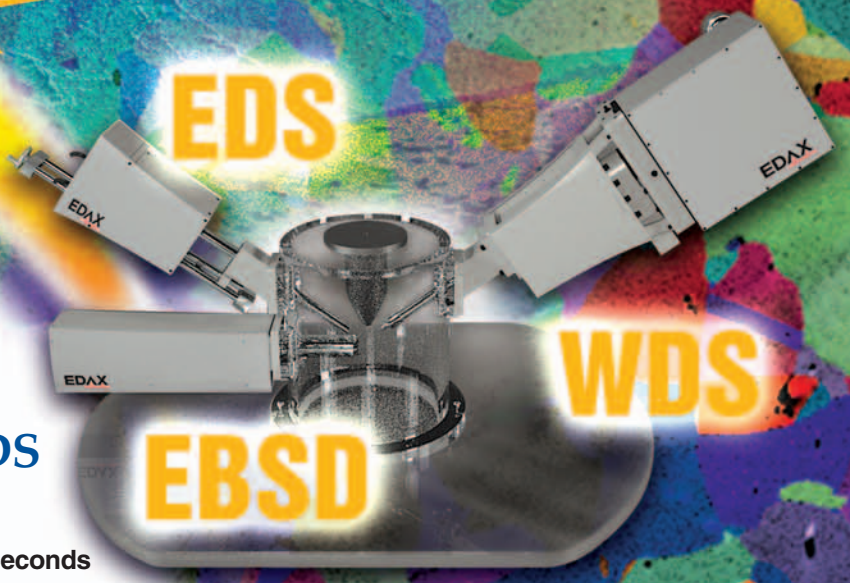


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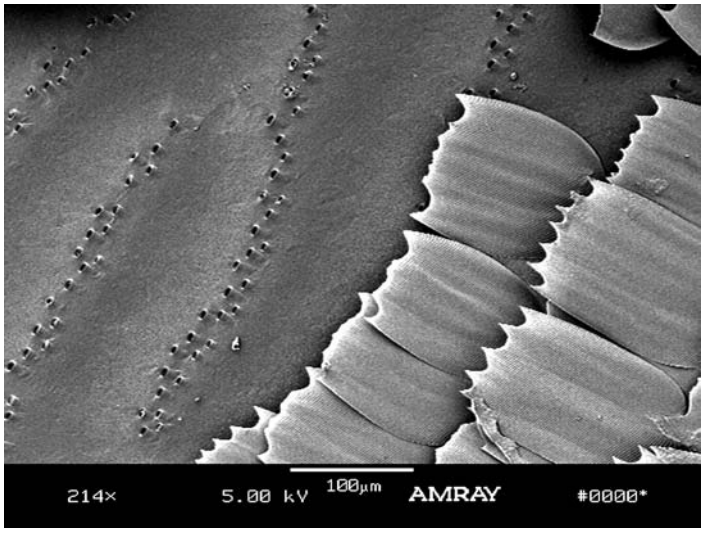


*Figure 2. The wings of a Noctuidae sp. (Moth) with a portion of the scales removed.*

## RESULTS

We used this technique to examine the cuticle of several different arthropods, including Theraphosidae (tarantulas), Apidae (bees), Scarabaeidae (beetles), and Noctuidae (moths). The fixation not only worked on the hairs of the different arthropods, but also worked on the scales of the moth. Because the majority of the specimens that were used had relatively high densities of hair, freeing the cuticles did not prove to be difficult. As previously stated, the hairs drew up the colloidal graphite toward the cuticle, thereby bonding the hairs tightly. Those specimens that were not as densely haired did not draw up the graphite as readily. More pressure was needed to make sure the specimen was firmly attached to the graphite. This often led to the graphite coming in contact with cuticle of the specimen. If this were the case, once the specimen was freed, the dried graphite on the cuticle was carefully flaked from the surface using a razor blade and fine forceps. This inevitably led to the deterioration of the cuticle, therefore the direct manipulation of the cuticle only occurred as a last resort. Once hairs were removed from the specimens, and the cuticles sufficiently freed of debris, they were gold coated and examined using an AMRAY Scanning Electron Microscope at 5kV.

Because there were no longer hairs blocking the cuticular surface, many features could then be examined that were previously not possible. Figure 1 a, b, c shows the result of this technique when applied to different arthropods. Hair densities (based on the number of follicles), boundaries and distances between hairs can be examined using this technique, resulting in images similar to those seen in Figure 1. In addition to working for arthropods with hairs, this method can also be used on insects with scales (Figure 2).

The use of this technique permits microscopic examination of regions on cuticular surfaces not normally accessible due to the dense mat of hairs covering surface architecture. ■

## ACKNOWLEDGEMENTS

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