A MICROPLASTICS RECOGNITION GUIDE

This guide is aimed at helping you recognise microplastics of interest under the microscope.

Foreword

Every analytical and scientific method has limitations. In recognising microplastics using this method, the major limitations you will encounter are of two types: instrumental and interpretative.

Instrumental limitations relate to your ability to observe the sample at magnifications, and in conditions, such that you can make your assessment with high level of confidence. A low-magnification microscope or a low-light microscope will diminish your ability to correctly identify particles as microplastics. These limitations can be overcome with higher quality instruments. Nevertheless, we invite you to acknowledge the limitations of the equipment you have on hand and to communicate these limitations to your students and see if anything can be done to tackle them. Often, students come up with very low-tech solutions that overcome or reduce the pre-existing limitations.

Interpretative limitations relate to your ability to interpret what you see under the microscope. These are related to the quality of the instrumentation (instrumental limitations), but also relate to the colour and morphology of the objects you will find under the microscope and the level of training you have completed to make your eyes and brain used to recognising certain shapes. These limitations are the easier to tackle, through training and recognition guides. Nevertheless, it is important to note that there will always be items that you see under the microscope that you will not be able to categorise. This is OK and is part of any scientific method of analysis.

Why spend so much time talking about fibres? Fibres are the major source of interpretative error in microplastics analysis. Fibres are the most common item found by microplastics researchers all over the world. They are also very present in nature, and this can cause confusion during analysis. It's important that you learn how to recognise naturally-occurring filaments and textile filaments (which are to be reported in the protocol). There are some distinguishing features of the various filaments. This guide will show you a few. Here below are some typical biases to be aware of. Students are very enthusiastic about discovery and often are driven by enthusiasm to incorrectly identify:

- all filaments with colour as man-made fibres (cellulose fibres like cotton may also be coloured),
- all filaments as man-made fibres,
- all naturally-occurring filaments as man-made fibres.

Observer bias (microfibre pollution is widely known) can only be countered through training. It is important that you spend some time with your students observing "positive control samples". These are samples you prepare with known items in them. It could be laundry water after washing a polyester vest/jumper, it could be wash water of a cotton cloth. This is something you can work out with your students.

FIBRES

Many objects in our environment appear filament-like. We typically identify these as fibres. Before we consider how to recognise fibres that are of artificial origin, which we report as microplastics, we need to learn to recognise fibres that are of biological origin.

Most fibres you will encounter are or were part of biological entities (some, very few, are mineral fibres, which we will ignore for now). Of this class of filaments of direct biological-origin are:

- Filaments that come from some types of plankton
- Cell and algal colonies
- Some cells
- Filament-like roots or fine branches, or parts of plants or plant matter
- Animal hair
- Animal-limbs (legs), antennae, etc.

Many of these can be identified as follows.

Plankton filaments – DO NOT REPORT as they are not microplastics

Often found attached to plankton, they are quite smooth and appear "glass-like" (colourless and transparent). The section often is tapered (from larger to finer). Sometimes they look like tubes.

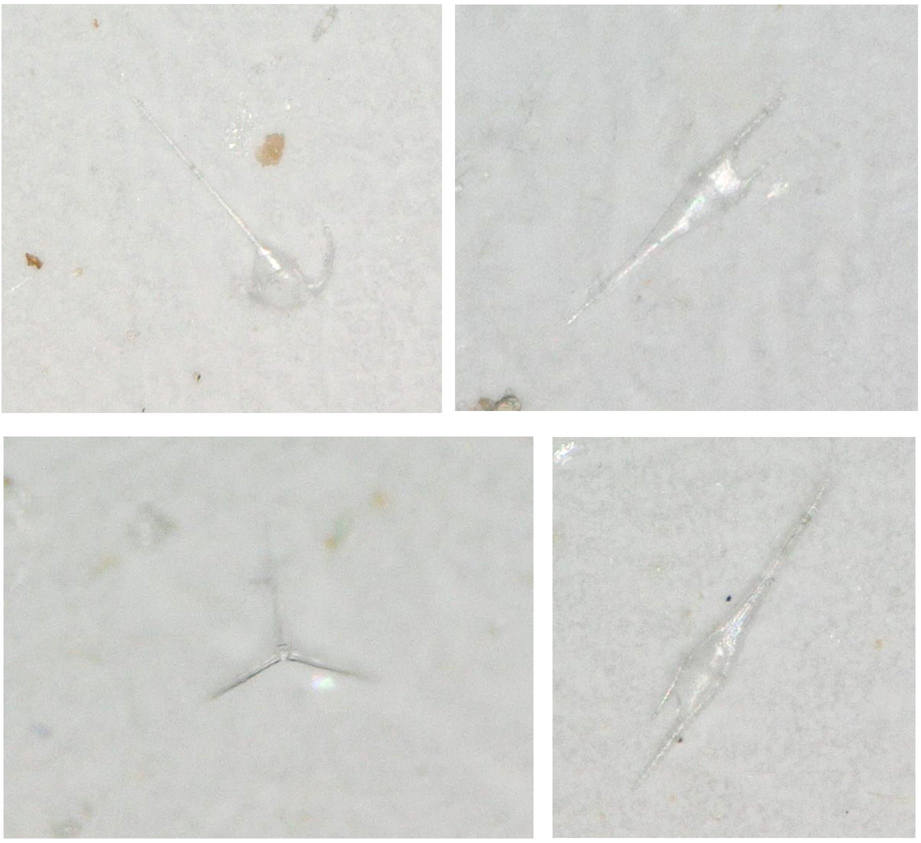


Figure 1. *Courtesy Deakin University. Various plankton and other biological shapes that you may find in surface waters show fibrous features. These are often mistaken for synthetic fibres or plastics, simply because they appear to be very regular, shiny and transparent (also colourless or green).*

Plant or animal origin biological filaments, algal colonies, cellular structures – DO NOT REPORT as they are not microplastics

Some plants have filaments that are lost in the water. These appear as very small roots or bits of leaf, etc. It is important to look at suspicious items carefully. Often naturally-occurring filaments like the ones below are brown-green, and appear “organic” in nature (wavey, irregular, etc.).

Some examples of what you can encounter are here below.

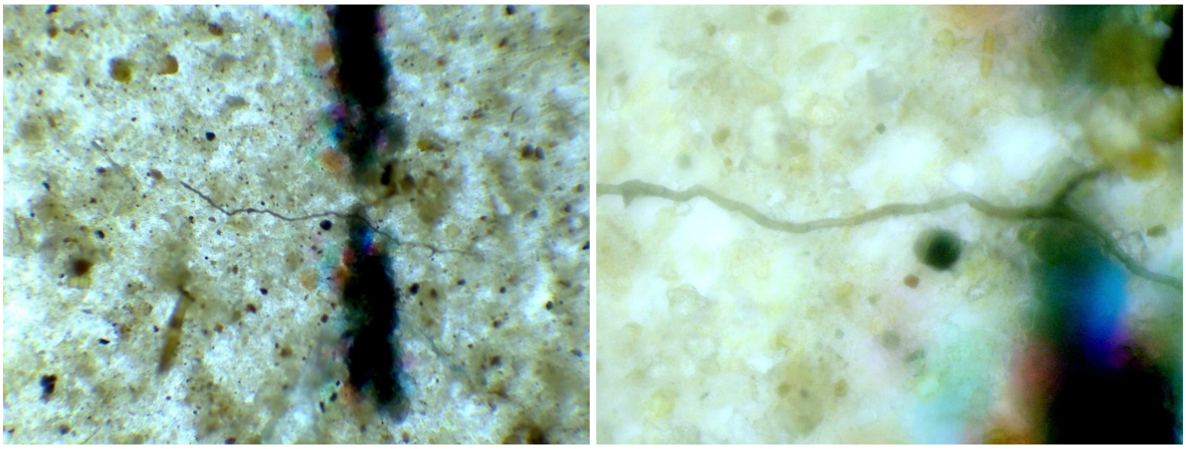


Figure 2. Courtesy ISS Ferraris Brunelleschi, Empoli, Italy. Green filament, with no visible cellular structure, but showing branched structure (low magnification on left, higher magnification of the same object on right).

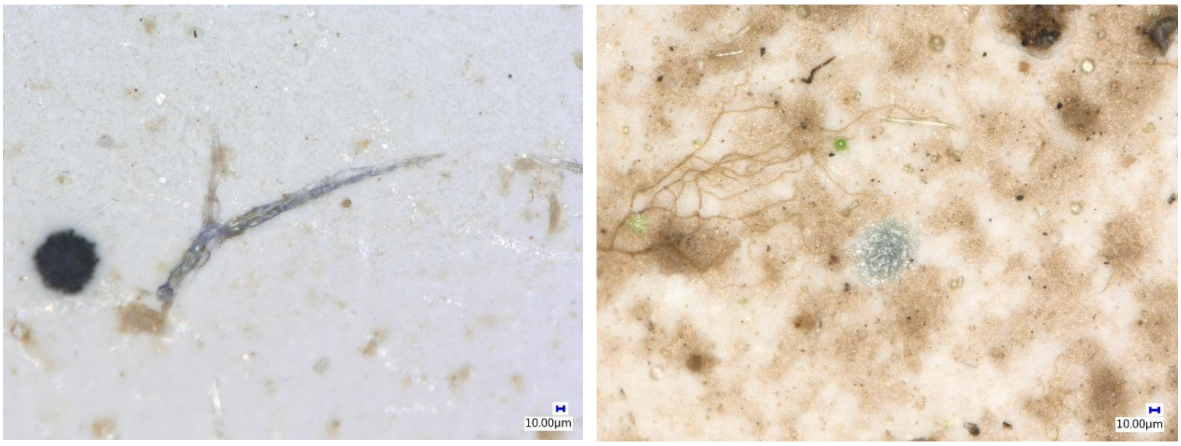


Figure 3. Courtesy Deakin University. Fibrous structures. Left: part of plant or animal. Right: branched filaments, probably of vegetable nature, next to a biological-origin blue-green shiny object and a round green algae.

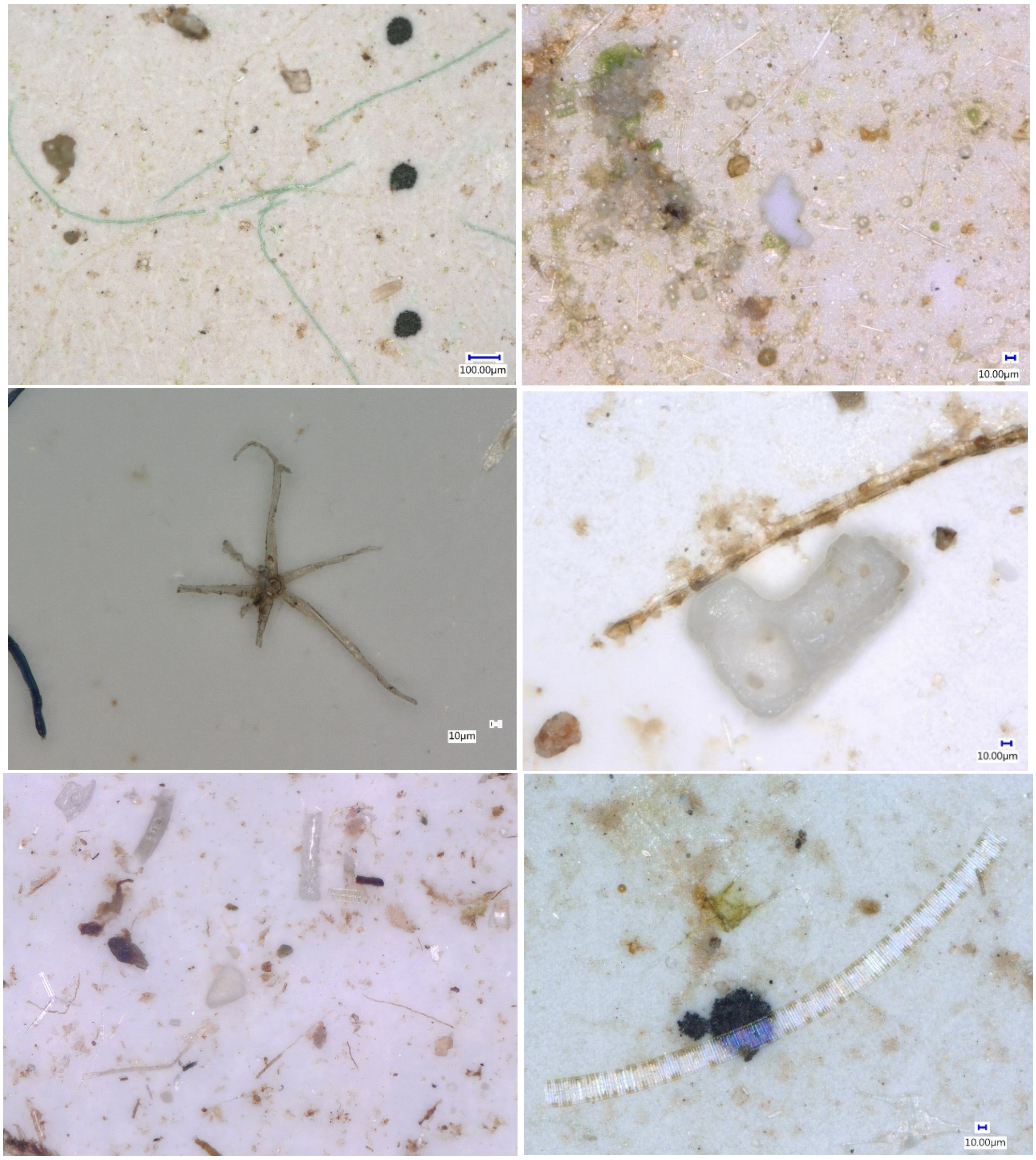


Figure 4. Courtesy of Deakin University. Examples of different fibres and filaments of biological origin and some debris that is of natural occurrence (tubules and other debris in bottom left image, sand grains in top right image). Top left: blue-green algae colonies in the shape of blue-green filaments. Top right: various naturally-occurring filaments (needle like shapes, in among many spherical algae). Second row, right: filament showing cellular structures (algal colony). Bottom right: algal colony in the shape of a filament.

NOTE: A typical school microscope has low resolution. It's not likely that you will see items like these with great clarity. One way you can determine whether they are of biological or man-derived origin is to look for how many "identical" or "very similar" objects there are in your sample. Fibres of biological origin will typically be abundant in any one sample. Man-made objects will be less common to find. So, if in doubt, if you have many objects that look very much identical, chances are they are of biological origin.

If in doubt, if you think an object shows cellular structures, or has ramifications (branched), consider it of biological origin.

Animal hair (non-textile animal hair) and plant debris – DO NOT REPORT as they are not microplastics

Many animals have fibres on their coat and sometimes on their wings (moths, butterflies, feathers, etc.). While there are many different types of small fibres that come from animals, here are some suggestions on how to identify those that you do not need to report as microplastics.

Many animal hairs have segmented structures (scales) on the surface, just like human hair, which allow us to identify their animal nature. They appear often dark brown or black under the microscope and have sometimes tapered section (thicker at one end and thinner at the other end, like a long cone).

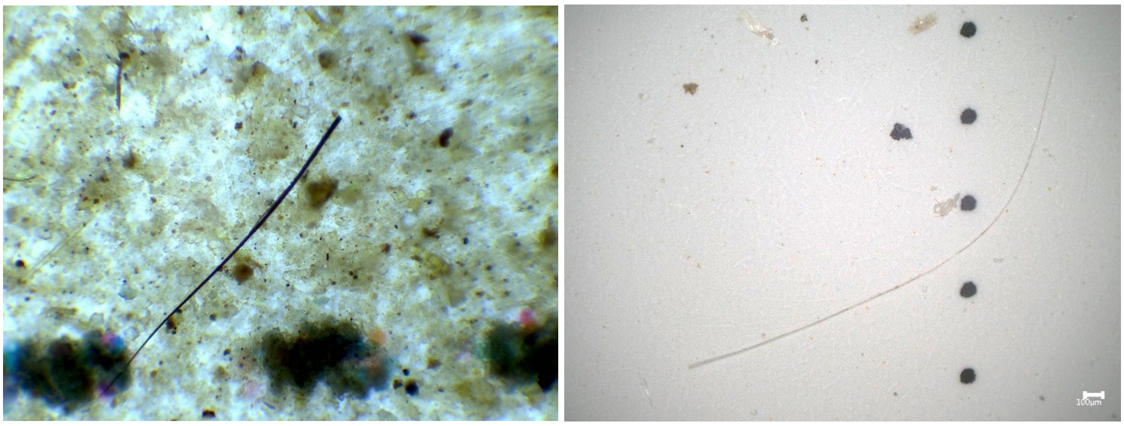


Figure 5. Left. Courtesy ISS Ferraris Brunelleschi, Empoli, Italy. Animal hair, with thinning section. Right. Courtesy of Deakin University, Australia. Both images show tapering section.

Some other animal-derived fibres may come from animal legs. Plant derived fibres may come from seeds, leaves, branches, pollens, etc. Some fibrous shapes have special features like spikes (like in the image that follows). These are very interesting items from a biological perspective. Sometimes, you will not be able to work out if a particular particle or filament is animal or plant derived.

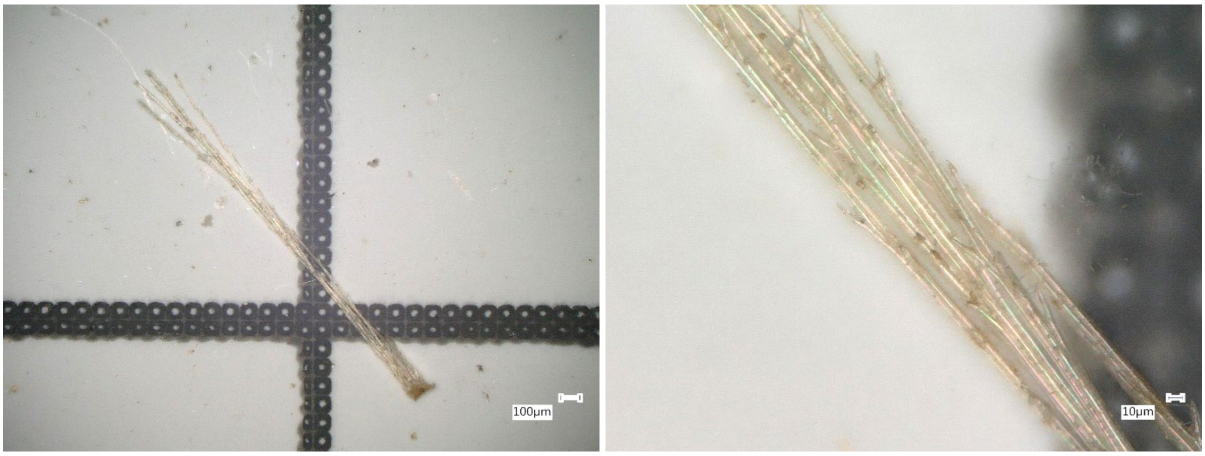


Figure 6. Courtesy of Deakin University, Australia. Part of an animal or plant (Left: low magnification, Right: high magnification).

Textile fibres – Cellulose – PLEASE REPORT THESE

There are many textile fibres that we use every day and are derived from plants. Some plants and fibres you might be familiar with are: cotton, jute, linen, hemp, wood pulp, etc. Cellulose itself is a biopolymer, but we report these fibres because textile processing typically adds chemicals, including plastic coatings, to cellulose fibres. So cotton and other cellulose-based fibres can become transporters of microplastics. We account for these fibres under the category of "natural fibres".

Cellulose fibres have a few common features under the microscope:

- They have a flat section (like a squashed straw or a ribbon)
- They appear like flat ribbons that twist irregularly. If your microscope is low resolution, you might see a fibre that bends at various points and has irregular section (diameter).
- They appear quite transparent, both when they are coloured and colourless. For instance, cotton fibres we see by eye as being white appear colourless and transparent under the microscope. This is just an effect of the microscope's magnification.
- The majority of cellulose fibres you will find are blue or pink-red, some are colourless.

When you see a fibre, ask yourself the following:

1. Could this be a fibre of biological origin (is it branched, does it show cellular structures? see paragraphs above)?
2. If not, does it look like a ribbon (flat section)?
3. If not sure, if your microscope does not magnify much, does the fibre section vary (is thinner in parts, then thicker again, then thinner) along the length?
4. If no, does this look like a round fibre (constant section)?
5. If no, does it become thinner at one end only (animal hair)
6. If no, does it show scales (use grazing light to help)?

Some examples from our Europe&Eurasia Trial:

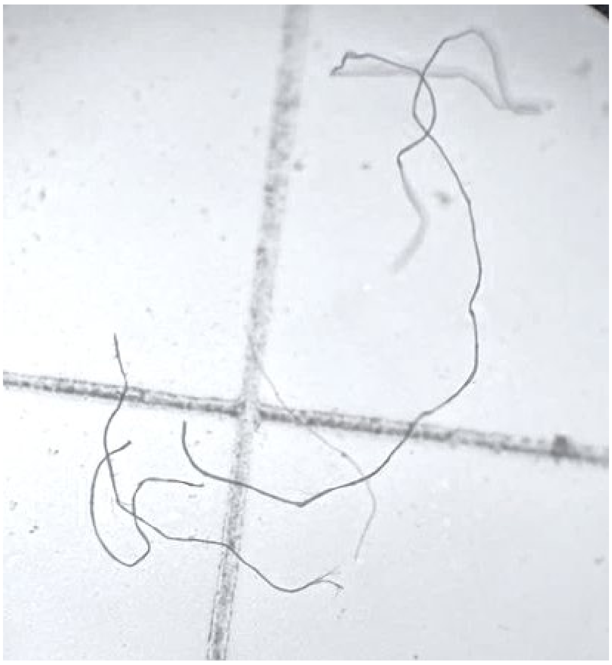



Figure 7. Courtesy IS Malignani Udine, Italy. Left: low magnification image of cellulose fibres. Right: higher magnification image of cellulose fibre. Note how these fibres are a little "crinkled" and twisted.

The fibres in Fig. 7 above show:

- In the left image: irregular bends, irregular section, somehow “sharp” kinks or crinkles
- In the right image: flat section, twisted-ribbon-like morphology.

These are common features in cellulose fibres. All cellulose fibres shown above and below have: irregular bends, irregular apparent section, some kinks (changes in directions, creases), twisted-ribbon-like morphology. These are common features that help you identify cellulose fibres.

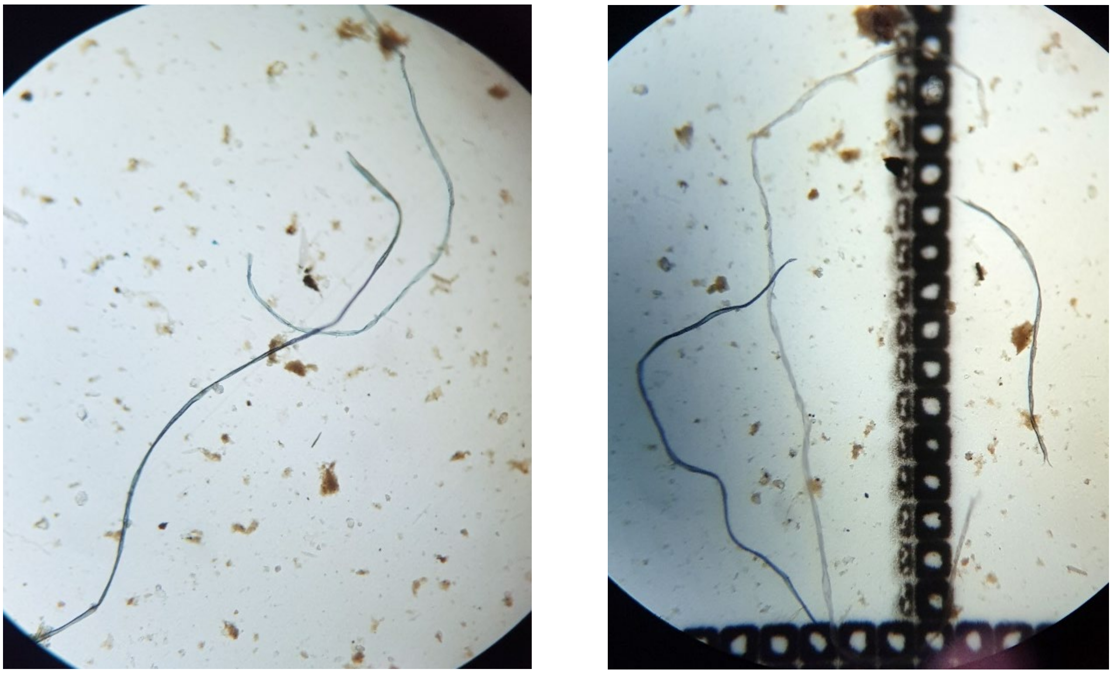


Figure 8. Courtesy Elementary School Banija, Karlovac, Croatia. Left: low magnification image of cellulose fibres. Right: slightly higher magnification image of cellulose fibres.

Often you will come across fibres that are long, such as in the previous images. Sometimes, though, you’ll come across fibre segments. Here are some examples.

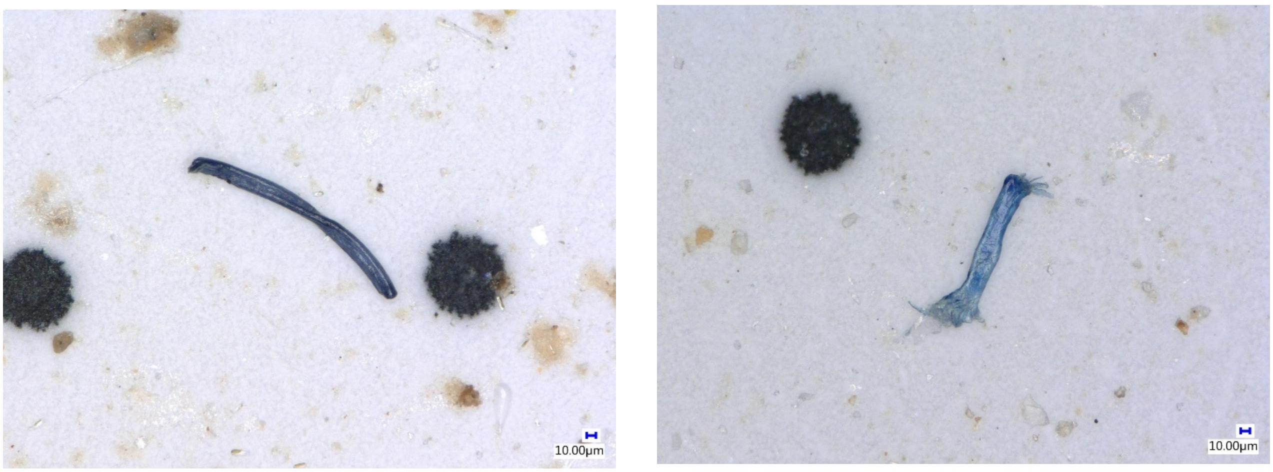


Figure 9. Courtesy Deakin University. Higher magnification images of cellulose fibre fragments.

Textile fibres – Wool and animal fibres (with scales) – PLEASE REPORT THESE

There are many textile fibres that we use every day and are derived from animals. Some fibres you might be familiar with are: wool, silk, alpaca, cashmere, etc. Animal-derived fibres are mostly composed of protein (keratin, silk fibroin and sericin, etc.). While the constituent proteins are natural and biodegradable, we report these fibres because textile processing typically adds chemicals, including plastic coatings, dyes, etc. to animal fibres. So textile animal fibres can become transporters of microplastics just like their cellulose counterparts.

We account for these fibres under the category of "natural fibres".

Aside from silk, which is excreted by silkworms as a continuous filament (just like spider silk, excreted by spiders), animal fibres are mostly animal hair, and therefore have finite length and a characteristic scale structure on the surface (just like human hair). This guide does not discuss silk in depth as it is very rare to find in surface waters and it is very difficult to distinguish from cellulose and man-made fibres given its flattened fibre structure. We will consider this a known experimental error that comes from interpretative limitations. Textile animal fibres, aside from silk, are pretty similar.

Animal fibres have a few common features under the microscope:

- They have a round section (like a cylinder, a rod)
- They have a constant diameter over the length
- They appear quite sinuous, with soft bends, as they are quite rigid. They can at times appear curly, with long curls.
- Sometimes the ends of a broken fibre appears fibrillated or with fluffy curls
- They appear quite transparent and shiny, both when they are coloured and colourless. For instance, textile animal fibres that we see by eye as being white appear colourless and transparent under the microscope. This is just an effect of the microscope's magnification.
- The majority of animal fibres you will find are blue or pink-red, some are really dark blue-black or green, and some are colourless.

You are not very likely to encounter many textile animal fibres in your work. Typically, for any 10-20 cellulose textile fibres you might encounter one textile animal fibre. This is similar for man-made fibres.

The microscope magnification and light source you use can make a big difference in how well you can identify certain fibres. A grazing light source helps a lot, when looking at fibres' surface morphology.

NOTE. It is often difficult to discriminate between textile animal fibres and man-made fibres. This is because animal fibres can appear quite solid and shiny under a microscope, with very regular diameter. It is therefore OK to identify fibres you encounter as either textile animal fibre (classified in the table as natural fibre) or as man-made fibre, if you are uncertain.

Figure 10 shows some examples of textile animal fibres found in surface waters, photographed using a metallographic microscope with lighting from above. Note the very faint scale structure and the solid-looking appearance. This homogeneous appearance and the shiny surface texture often draw the students to identify these fibres as man-made.

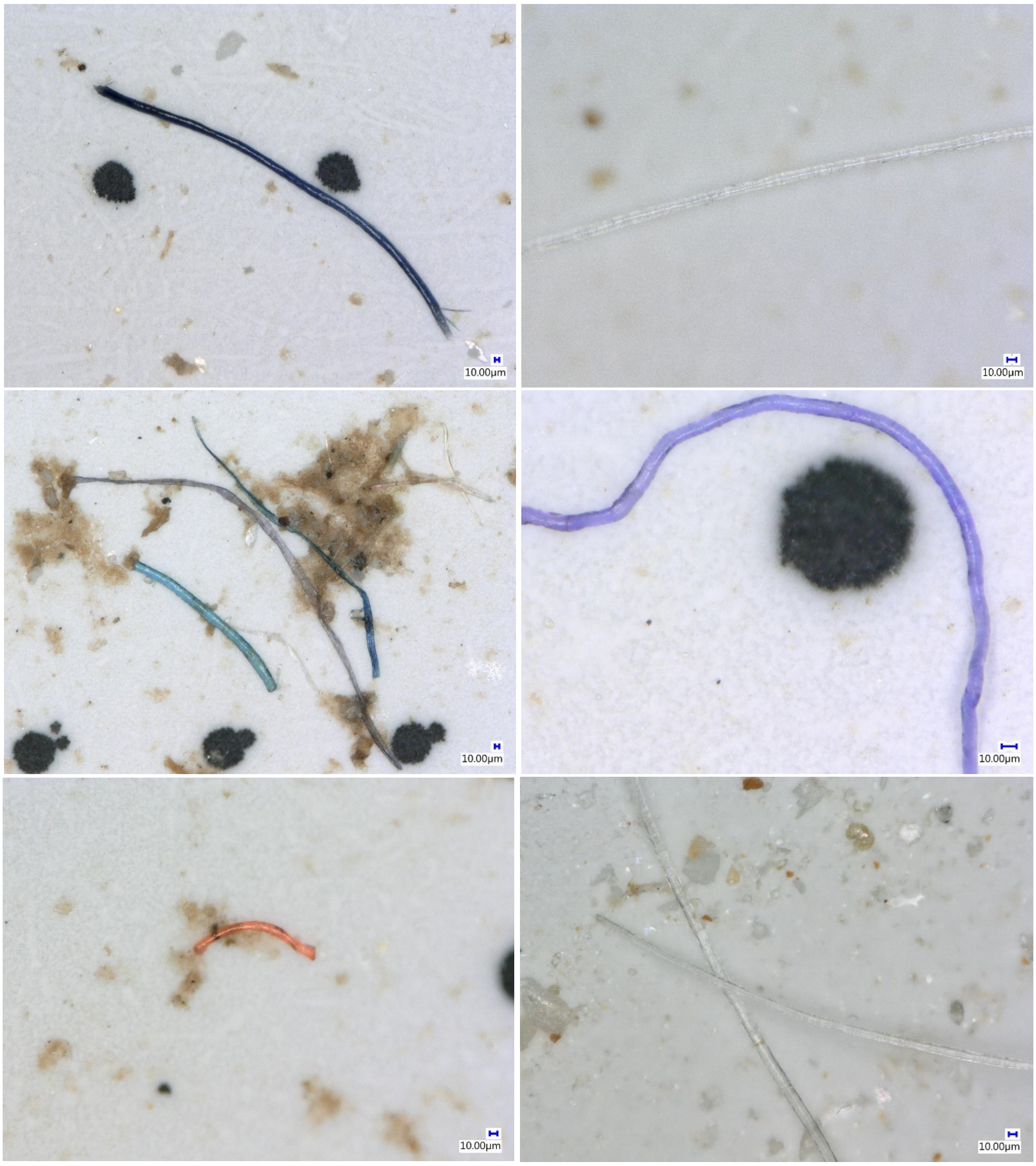


Figure 10. Courtesy Deakin University. Higher magnification images of textile animal fibre fragments. Middle row left image: light blue textile animal fibre next to two (grey and blue/green) textile cellulose fibres.

Textile fibres – Man-made fibres – PLEASE REPORT THESE

There are many man-made textile fibres that we use every day and are composed of renewable, degradable (viscose, rayon, lyocell, from cellulose that has been treated to become insoluble; bamboo, also from cellulose) or synthetic and non-degradable components (nylon, elastane (polyurethane), polypropylene, polyester (PET)). Some fibres you might be familiar with are: polyester, viscose, elastane. These fibres can be categorised as primary microplastics, even if some are degradable in the long term. The coatings that might be on their surface might also be contributing to microplastics pollution. You could say that man-made fibres might be contributing twice: themselves and the coatings they may carry.

The two major sources of all textile fibre pollution in water bodies are: laundering and general use. Man-made fibres are not abundant in most surface water samples. It is believed that this is mostly due to their density, which is typically greater than that of salt and fresh water. Therefore, you should be able to find more man-made fibres in either agitated water bodies (at the edge of the beach/sea) or in the sediment.

What to look for: solid-looking fibres, with regular diameter. Many synthetic fibres can look gummy, or “plasticky” when you shine a light at low angle. Look for very shiny, regular surfaces, or regular grooves on the fibre surface. The most common sections are: square, rectangular, round, flat with channels. The edges of man-made fibres can also look weird. Some end with a bulge, some end like a cut noodle. Typically, man-made fibres have non-fibrillated, non-frayed, ends.

Man-made fibres are very different from cellulose fibres. Sometimes, though, you may identify textile fibres of animal origin as a man-made fibre. That is fine, it’s just an example of interpretative uncertainty. If you have any doubts, do identify the fibre as man-made. It’s a safe assumption to make.

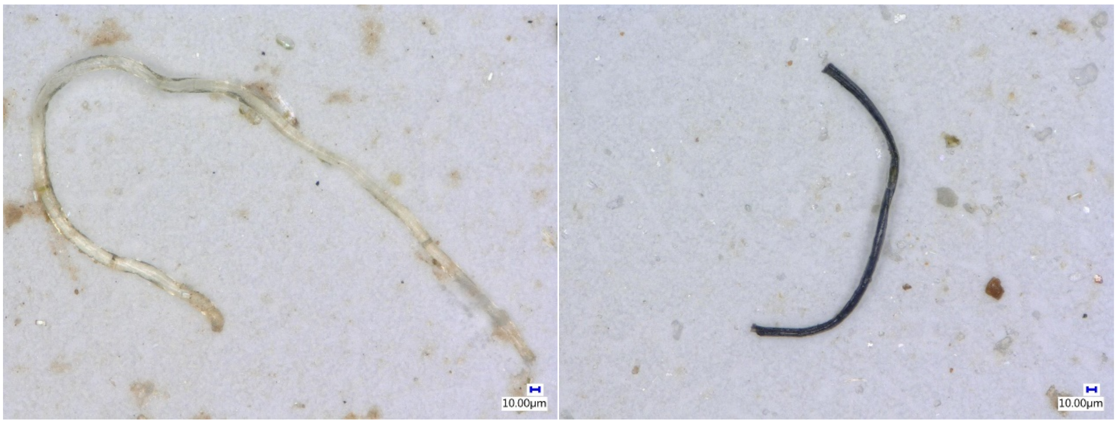
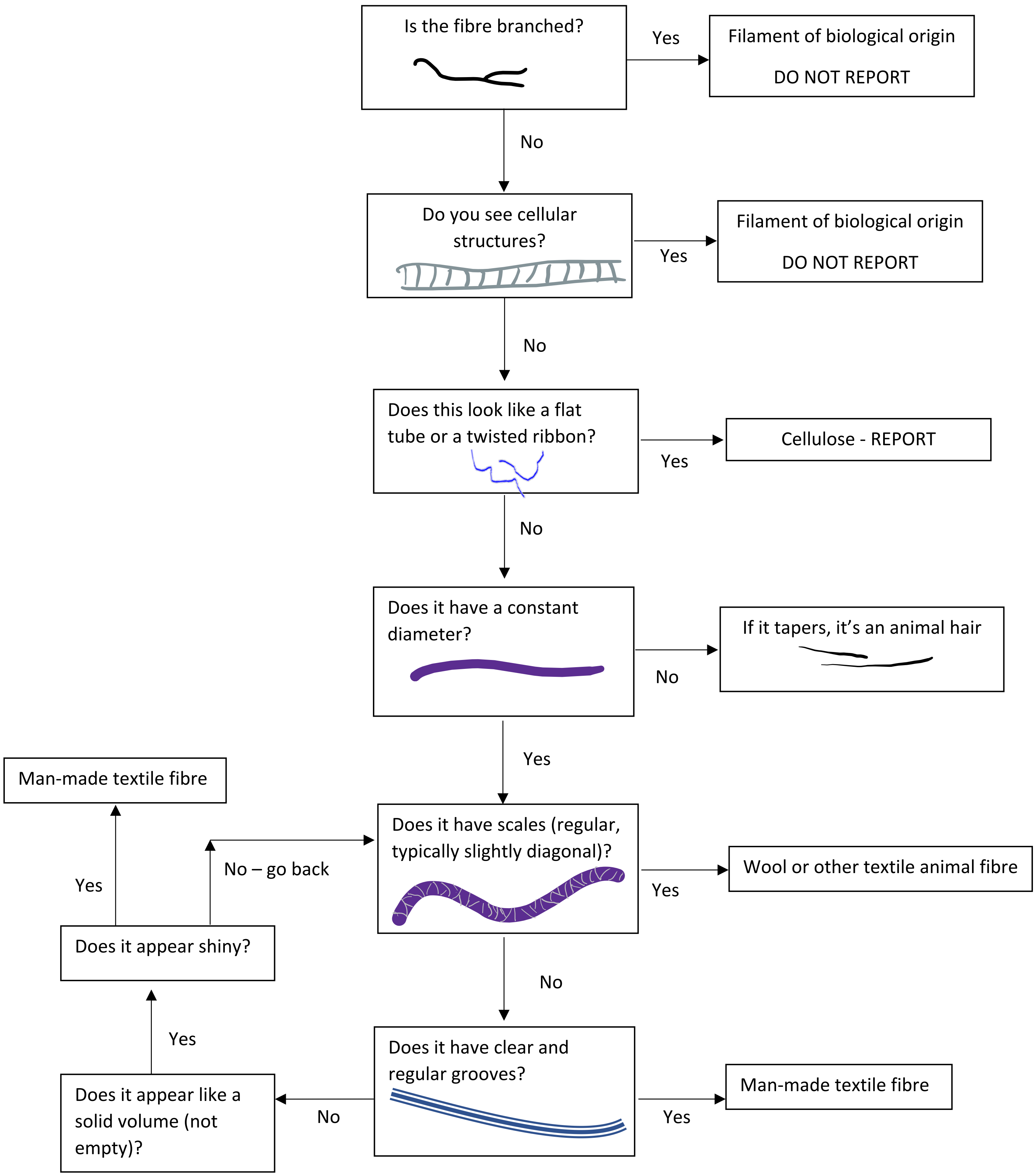


Figure 111. Courtesy Deakin University. Higher magnification images of textile man-made fibre fragments. Left: round section, right: flat rectangular section with a slight groove

Figure 122. Courtesy Deakin University. Higher magnification images of textile man-made fibre fragments. Top left: one round section man-made fibre, next to a cellulose fibre, both colourless. Top right: flat rectangular section with grooves. Bottom right: round section fibre next to a colourless piece of microplastic. Bottom right: round section fibre with one cut end (neat cut on one end and semi-melted end.

When you see a fibre, ASK YOURSELF:



PARTICLES, SHEETS and FRAGMENTS

Many objects that are naturally occurring in our surface waters appear like they could be pieces of plastic when you see them under a microscope. Many are fragments of plant or animals, they could be grains of sand. Before we consider how to recognise microplastics, we need to learn to recognise particles that are of natural (biological or mineral) origin.

Most particles, sheets and fragments you will encounter are or were part of biological or mineral entities. Things that belong to this class are:

- Plankton, including algae (including diatoms) and algal colonies
- Fragments of cells, animals, leaves, plant matter
- Animal hide, shells, etc
- Grains of sand
- Microorganisms including micro-invertebrates

Many of these can be identified as follows.

Microplankton and biological-origin shapes – DO NOT REPORT as they are not microplastics

Microplankton is a collective noun that describes many different species of living organisms you may find in surface waters. Plankton is everywhere, but every water body may have a different proportion of many different types of organisms.

What characterises microplankton are the following:

- Abundant presence of shapes that look very similar to each other
- Colourless, iridescent or, if coloured, green/green-blue or brown (rarely red)
- Very regular shapes

The most typical microplankton shapes you may encounter are:

- Spherical, often misidentified as plastic microbeads
- Segment (cube-like)
- Star-like
- Lenticular (lens cross-section shape)
- Filament (as discussed in the earlier fibres sections)

Many components of microplankton are quite smooth and appear “glass-like” (colourless and transparent), some appear like “alien” or “space-like” geometrical shapes. Remember, that you will be limited by the magnification of your microscope in what you’ll be able to recognise.

One way to tell if something is a microplastic or microplankton is how many items that are identical or very similar do you have in your sample? If you have many similar or identical items, then it’s more likely that you are looking at microplankton, not microplastics.

The following images contain a mixture of microplankton and other naturally occurring debris in shape of particles, sheets and fragments. Sand will be presented separately.

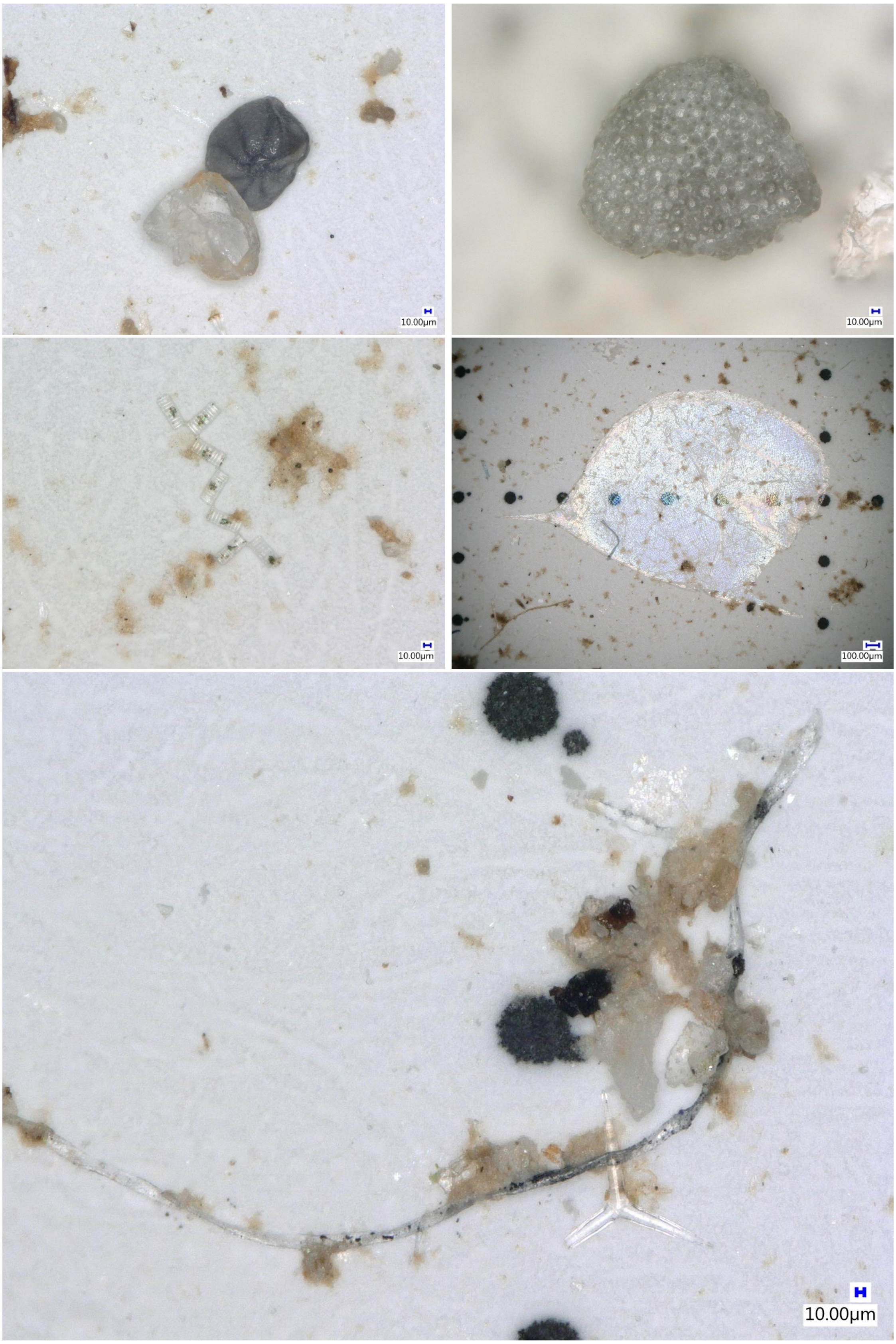


Figure 133. Courtesy Deakin University. Higher magnification images of naturally occurring shapes. Top row: undefined bio-fragments (likely sponge on the right). Middle row: left: algae colonies; right: scale or other animal-hide debris. Bottom image: star-shaped microplankton next to cellulose textile fibre and other naturally occurring debris.

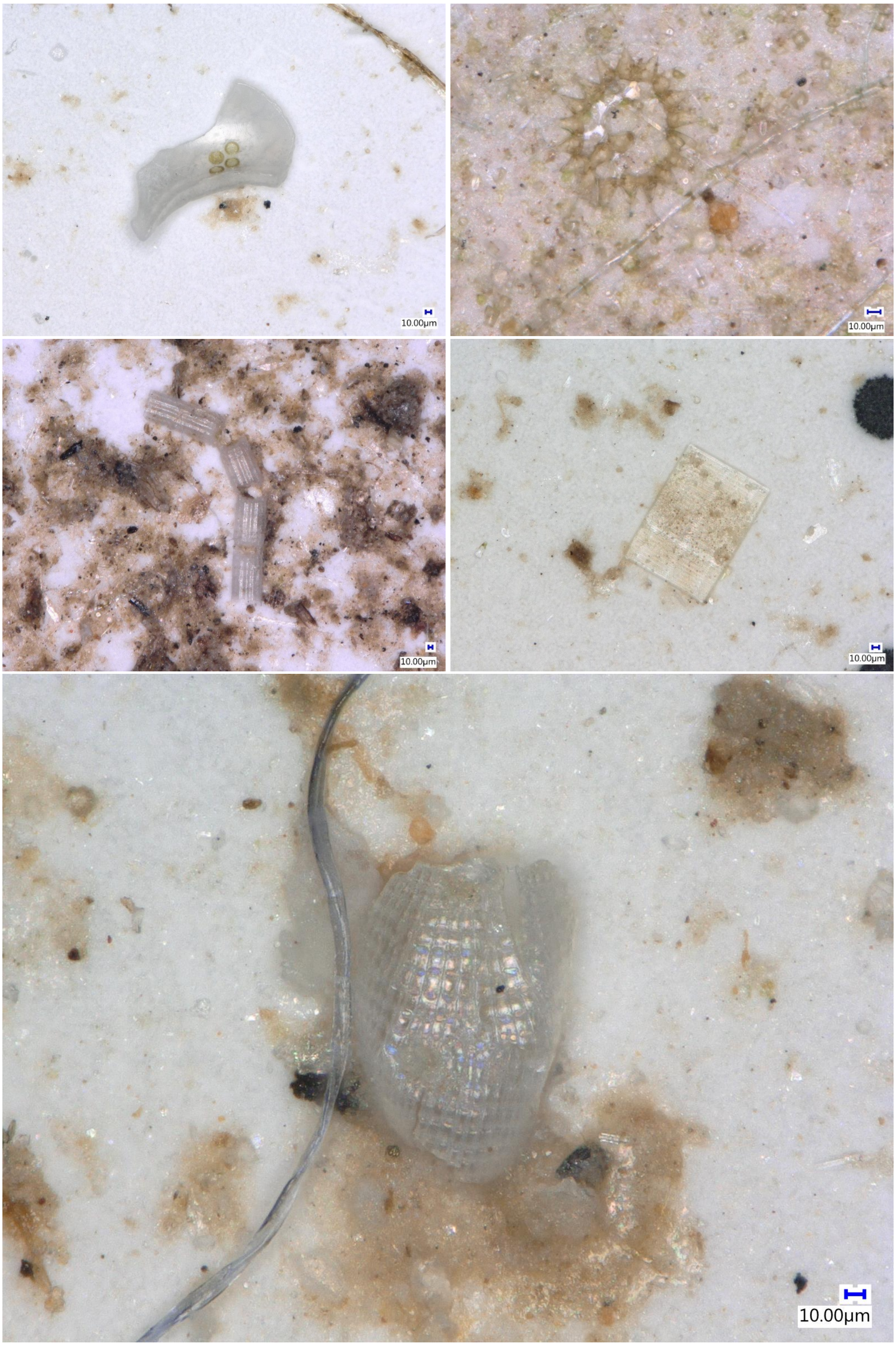


Figure 144. Courtesy Deakin University. High magnification images of naturally occurring shapes. Top row: left, undefined bio-fragment (with 4 algae on surface); right, round algae simplex colony. Middle row: algal colonies. Bottom image: scale or other animal-hide debris next to cellulose textile fibre and other naturally occurring debris.

Figure 155. Courtesy Deakin University. High magnification images of naturally occurring shapes. Top row: left, animal hide fragment; right, animal or plant debris. Middle row: left, animal or plant debris, right animal-hide debris in amongst a sea of round algae (green round shapes). Bottom image: microplankton next to naturally occurring debris (fibrous, plant-derived).

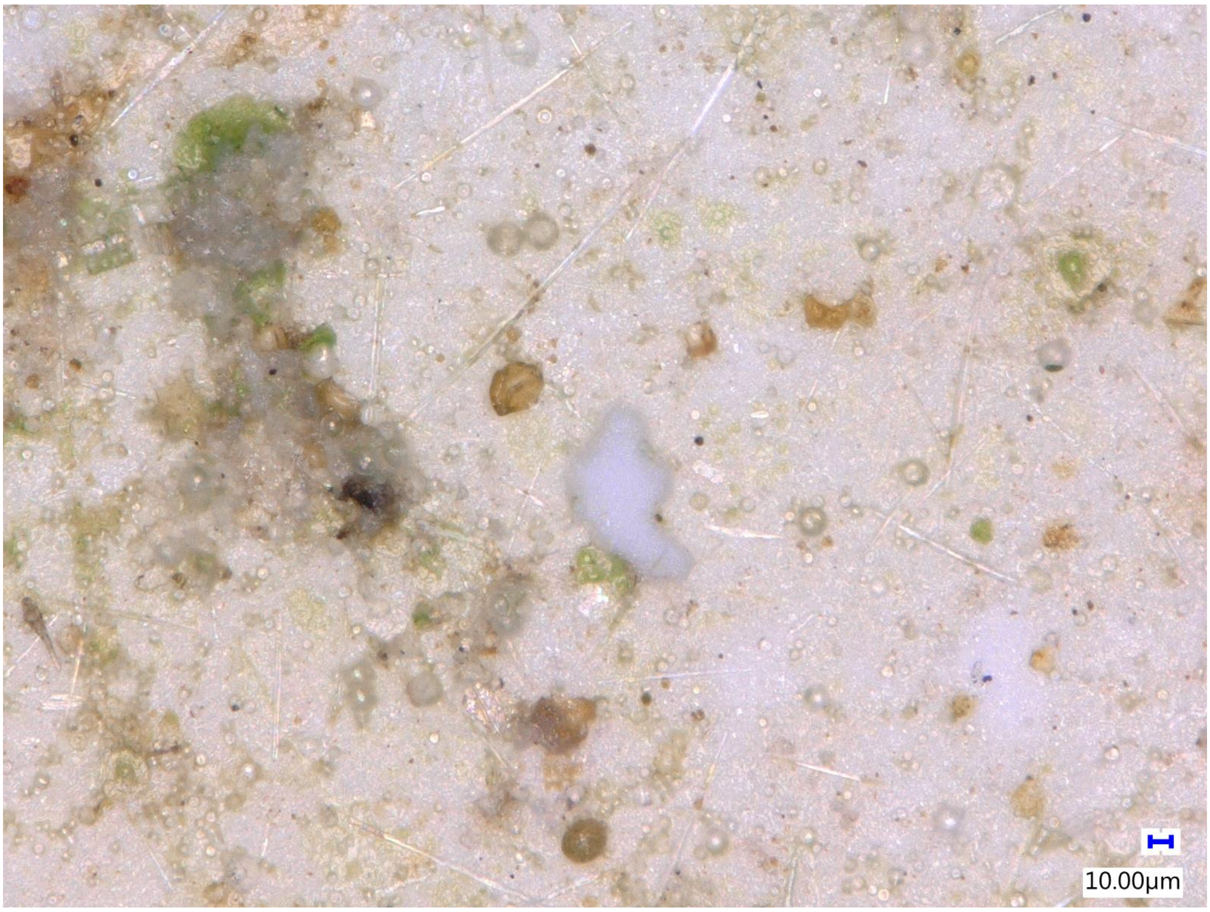


Figure 166. Courtesy Deakin University. High magnification images of naturally occurring shapes and a suspicious white particle (thought to be microplastic – marked as unknown). This image shows a rich microplankton with shapes that are needle-like, spherical and rectangular (top left quarter). This image also shows some gelatinous shapes (top right quarter) with a green centre, or larger gelatinous shapes (top left quarter). These are biological shapes.

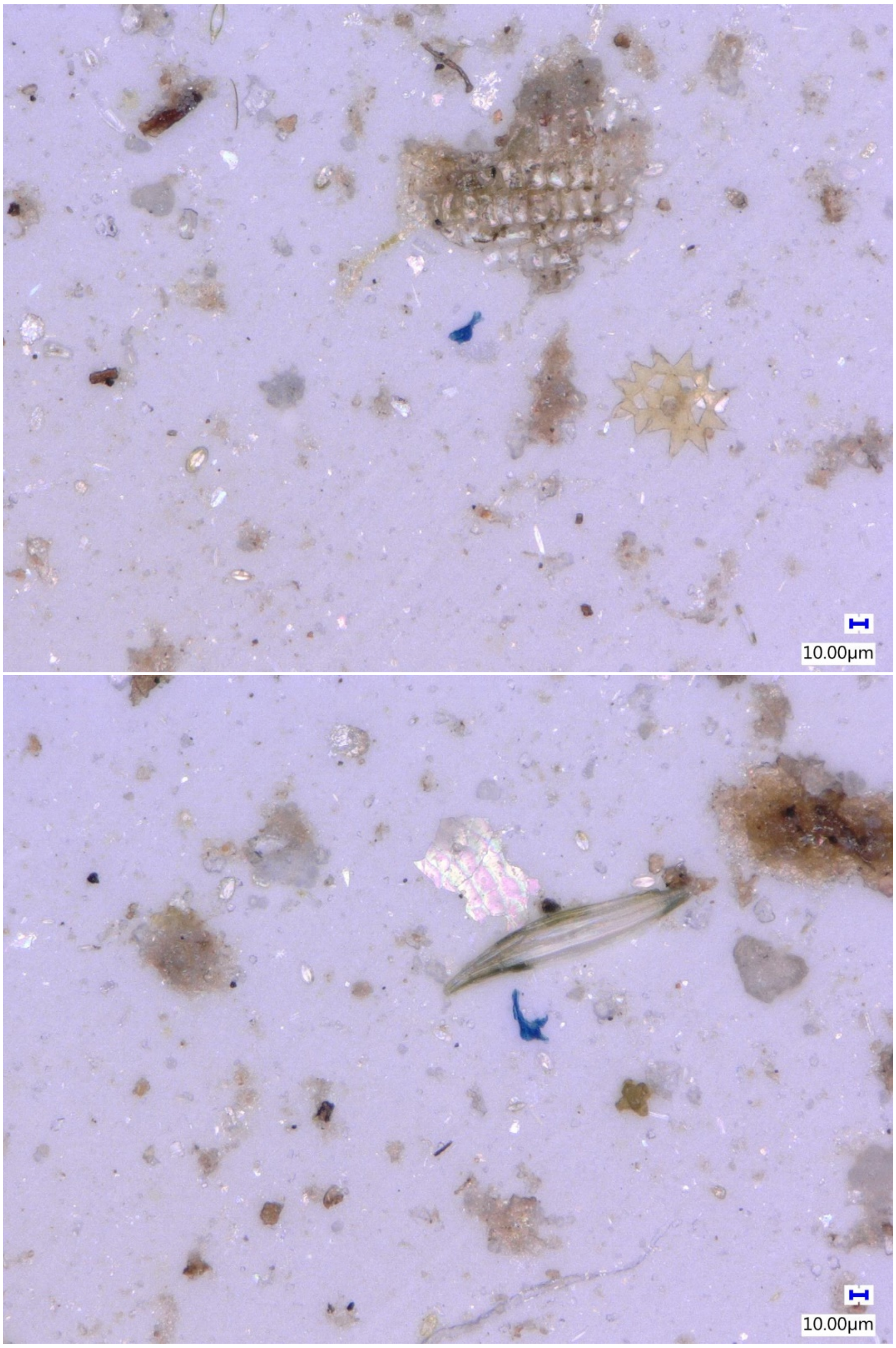


Figure 177. Courtesy Deakin University. High magnification images of naturally occurring shapes and two blue particles (microplastics). These images show microplankton next to microplastics, highlighting how the size of microplastics often matches that of microplankton. These images also show some other biological debris of undefined shapes (shiny flake in bottom image).

Sands and other inorganic debris – DO NOT REPORT as they are not microplastics

Sands and other inorganic solids may appear like microplastics to the untrained eye. A simple way to assess whether a particle is sand or microplastic is to see how many similar particles there are. If there are many, then the particle is likely sand.

Sands are made of a range of particles that can have very nice geometrical shapes (biosilica, etc.) as well as less defined shapes. Figure 16 shows a typical example of debris that contains regular-geometry (bio-derived) shapes as well as rock fragments (irregular shapes).

Sands may appear partially transparent under the microscope and have typically earthy colours. It's important that you look at the water body you are sampling, and maybe also collect some of this dense sediment when sampling (or the sand on the banks of the water body), to ensure your students can compare the sediment with what they have found on the filter.

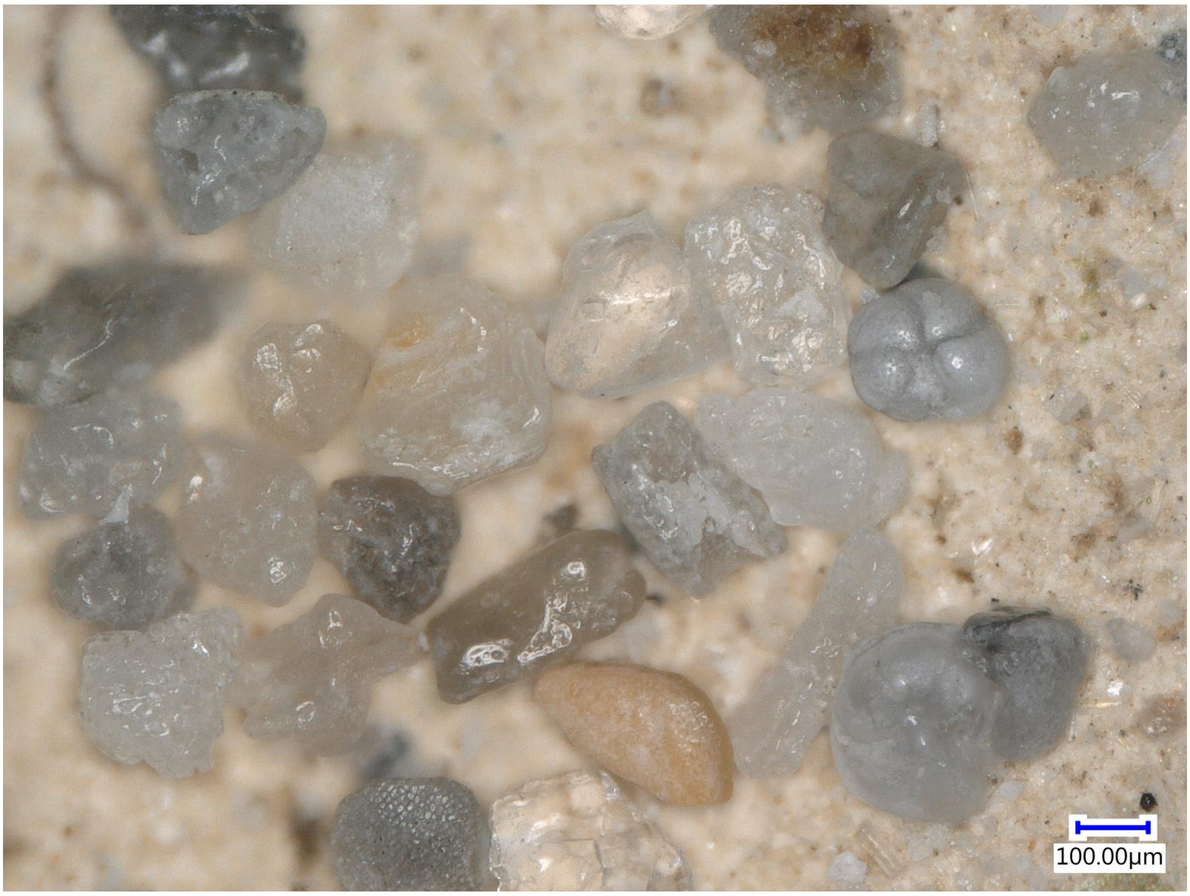


Figure 18. Courtesy Deakin University. High magnification images of naturally occurring sand shapes. Some of these shapes are clearly bio-derived (regular patterns on the surface and geometry) while some are just rock debris.

Plastic particles – Please REPORT as they are microplastics

There are four main concepts that relate to plastic particle identification.

The first. Plastics can take many shapes, especially when small. That's why it's important that you first learn to recognise what things are not microplastics. Sometimes, though, it's difficult to identify particles with high certainty. It's OK, make your best guess.

The second aspect that is important to note is that you will not be able to correctly identify all plastic particles in your samples. This is OK and it is taken into account as a limitation of the protocol. Don't spend excessive amounts of time to make sure you correctly identify all particles. Rather, try to look at a greater number of filter membranes.

The best thing to do is, therefore, to look for colour first. If you see something blue, purple, red, green or yellow, take a closer look. It could be a plastic particle, it could be part of a coating (look for colour spots from inkjet printing or print evidence (see Fig. 17). Most particles identified by optical methods are blue and purple. Early trials in Europe-Eurasia yielded the same result. If you only report coloured particles, it is not a problem. This is because the volume of plastics produced in the various colours is known, and therefore we can extrapolate the overall number of plastic particles likely to be in bodies of water (or at least their relative volume) for the other colours.

The third aspect is that it's OK to identify "suspicious" particles as "unknown" items. It is important for the team to know how difficult it can be to identify plastic particles.

The fourth aspect is size. Most particles you find will likely be 200 micron or smaller. Look closely.

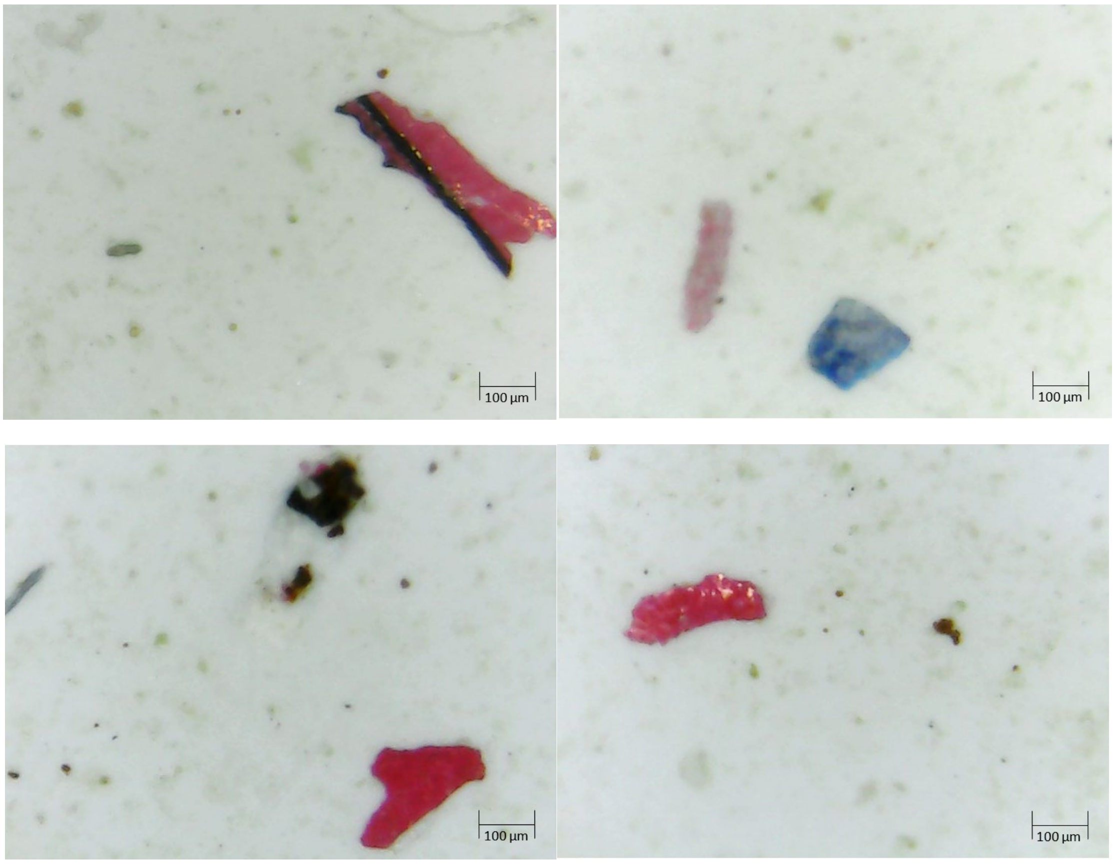


Figure 189. Courtesy Uzorak 1-OŠ Šime Budinića Zadar, Croatia. Particles which are likely plastic. Note the colour and the jaggered edges.