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**REPORT**

# Filamentous Biological Entities Obtained from the Stratosphere

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We previously reported the presence of large, non-filamentous, biological entities including a diatom fragment in the stratosphere at heights of between 22–27km. Here we report clear evidence for the presence of filamentous entities associated with a relatively large particle mass collected from the stratosphere. Although viable fungi have previously been isolated from the stratosphere, this is the first report of a filamentous microorganism being observed *in situ* on a stratospheric particle mass.

## Introduction

We previously reported the presence of large biological entities in the stratosphere at a height of between 22–27km (Wainwright *et al.,* 2013a, b). The biological entities so discovered were often unusual and difficult to ascribe to biological forms found on Earth, an exception being provided by the isolation, from the stratosphere, of a clearly defined fragment of a diatom frustule (Wainwright *et al*., 2013a). In our previous work however, we have not seen clear, irrefutable evidence for filamentous, biological entities, such as filamentous bacteria or fungi associated with particle masses so large that they could not have been plausibly argued to be lofted from the ground. Viable fungi have however, been isolated from the stratosphere (Imshenetsky *et al.* 1978; Wainwright, *et al.*, 2003; Wainwright *et al.* 2004; Wainwright *et al.*, 2006, Wainwright, 2008) showing that it should be possible to find evidence for the presence of fungal hyphae and mycelium in this region. Here we report the occurrence of a filamentous biological entity associated with a relatively large particle mass collected from the stratosphere.

## Materials and Methods

A balloon-launched sampling device was released from Chester, NW England on 31,7, 2013. The sampler included a drawer mechanism that could be automatically opened and closed at any desired height. The stratosphere sampler carried a video camera by which the opening and closing of the sampling drawer could be viewed, confirmed and recorded. The sampling apparatus was protected from downfall of particulate matter from the balloon by a cover. Prior to launch, the inside of the draw device was scrupulously cleaned, air blasted and finally swabbed with alcohol. New scanning electron microscope stubs were placed in rows inside the drawer with their top surfaces facing outwards so that when the draw was opened any particulate matter in the stratosphere would attach to them and they could later be removed for examination under the scanning electron microscope. The protective layer on the surface of the stub was peeled off just before launching under a cover to prevent any particulate contamination. After sampling, the apparatus was transported to the laboratory and opened under conditions which avoided exposure of the stubs to any contaminating dust and the stubs were similarly transferred under cover to the scanning E/M. The stubs were then sputter-coated with gold for 30secs at 30mA and then examined using a SEM (JEOL 6500F).

### *Balloon launch*

The balloon was launched from an open field near Dunham on the Hill (near Ellesmere Port, Cheshire, England) during daylight hours and traversed to just south of Wakefield in West Yorkshire (England). The sampling drawer was opened for 17 minutes as the balloon rose from 22026m to 27008m. The sampling apparatus was returned to Earth (by parachute) undamaged and completely intact.

### *Control flight*

A separate control flight was made to the stratosphere prior to the sampling flight, when the draw was not opened, but all other sampling procedures were observed. No particulate matter was found (using the SEM) on any of the unexposed microscope stubs, showing that the draw remained airtight and that none of the stubs was exposed to particles at, or near, ground-level or at any height up to the stratosphere. These results also show that no particles contaminated the stubs during any of the sample

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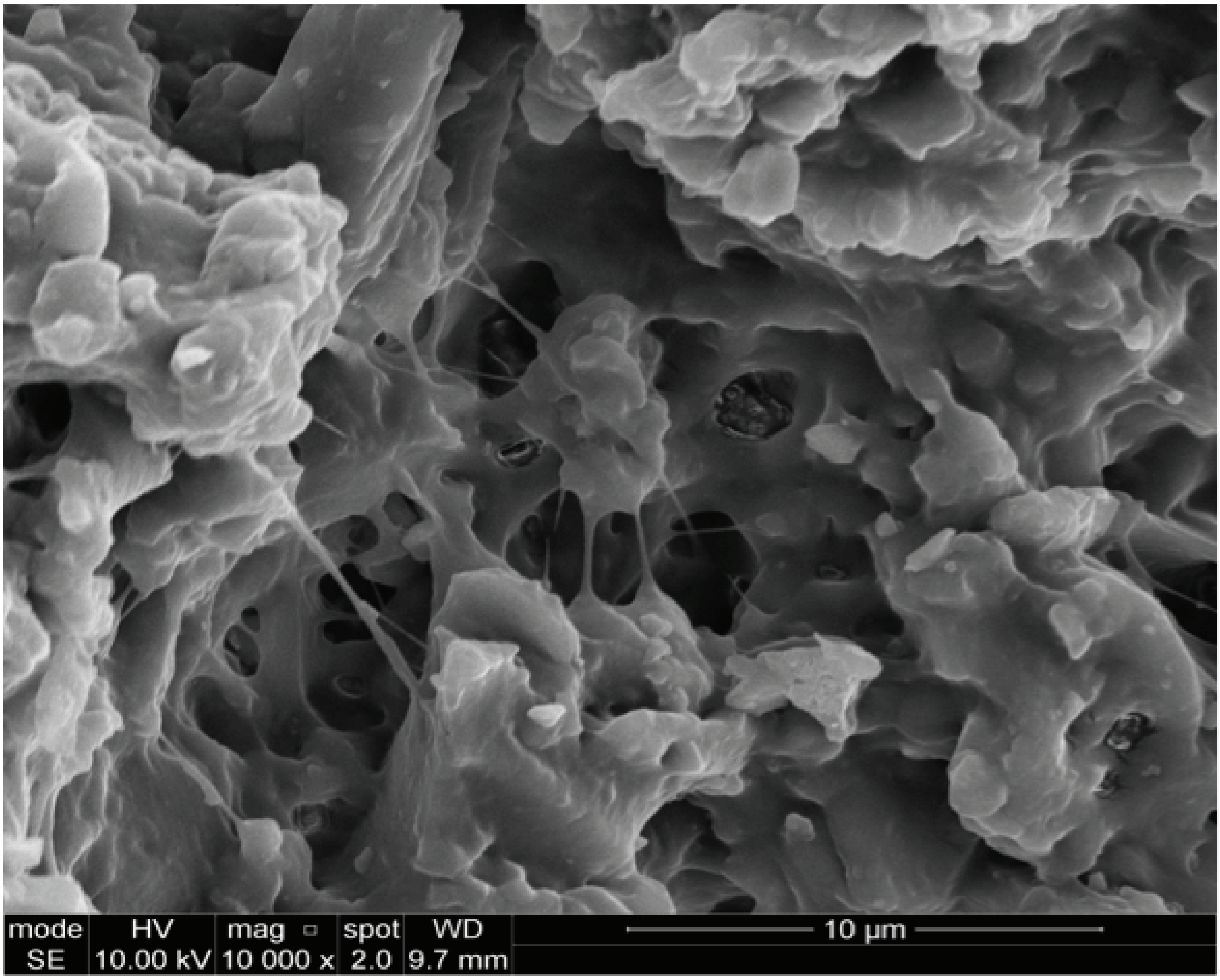


**Fig. 1:** Detail of a large particle mass isolated from the stratosphere showing microbial filaments, some of which show branching.

processing procedures, thereby demonstrating that the scrupulous procedures used to prevent ground level contamination proved effective and that no such contamination occurred.

## Results of Electron Microscopy

**Fig. 1** shows a detail of a particle mass sampled from the stratosphere of size around 200 micron. Long threads can be seen throughout the central part of the image, some showing signs of obvious branching; the filaments in the central region of the photograph also originate from what appears to be a mass of bacteria covered in slime material, suggesting that the filaments are bacterial. EDX analysis (**Fig. 2**) shows that the filaments are made of carbon and oxygen only and there are no signs of the presence of inorganic ions, such as calcium and silicon; particles of which might simulate biological entities. It is obvious from both **Fig. 1** and **Fig. 2** that the filaments show signs of flattening, and effect typically seen when filamentous microorganisms are observed under the scanning electron microscope. Such flattening might also be caused by the low pressure effects resulting from residence of the filaments in the stratosphere. The obvious conclusion which can be drawn from **Figs. 1** and **2** is that the stratospheric particle contains a filamentous organism which could be a fungus or a filamentous bacterium, e.g. *Nocardia.* Although viable fungi have been isolated from the stratosphere and have been grown and identified in the laboratory, this is the first report of filamentous microorganism being observed *in situ* in a particle isolated from the stratosphere.

We have discussed previously the question of contamination from the sampling balloon and atmosphere during sampling and have emphasised that a camera was attached to the sampler to confirm that the sampling drawer opened and closed in the stratosphere, and that no particles whatsoever were collected on a control sampling trip, when it was confirmed that the drawer remained closed throughout the balloon-sampling flight (Wainwright *et al.* 2013a, b). The coarse grain mineral background in **Fig. 1** (similar to interplanetary dust) clearly shows that the host mass for the biological structures is not a piece of contaminating laboratory dust. We are confident then that the biological filaments shown in **Figs. 1** and **2** originated from the stratosphere at a height of between 22–27Km. It is noteworthy that contamination was invoked by critics to explain our earlier findings of the presence of both bacterial clumps in the stratosphere and the isolation of viable bacteria and fungi from a height of 41km. The

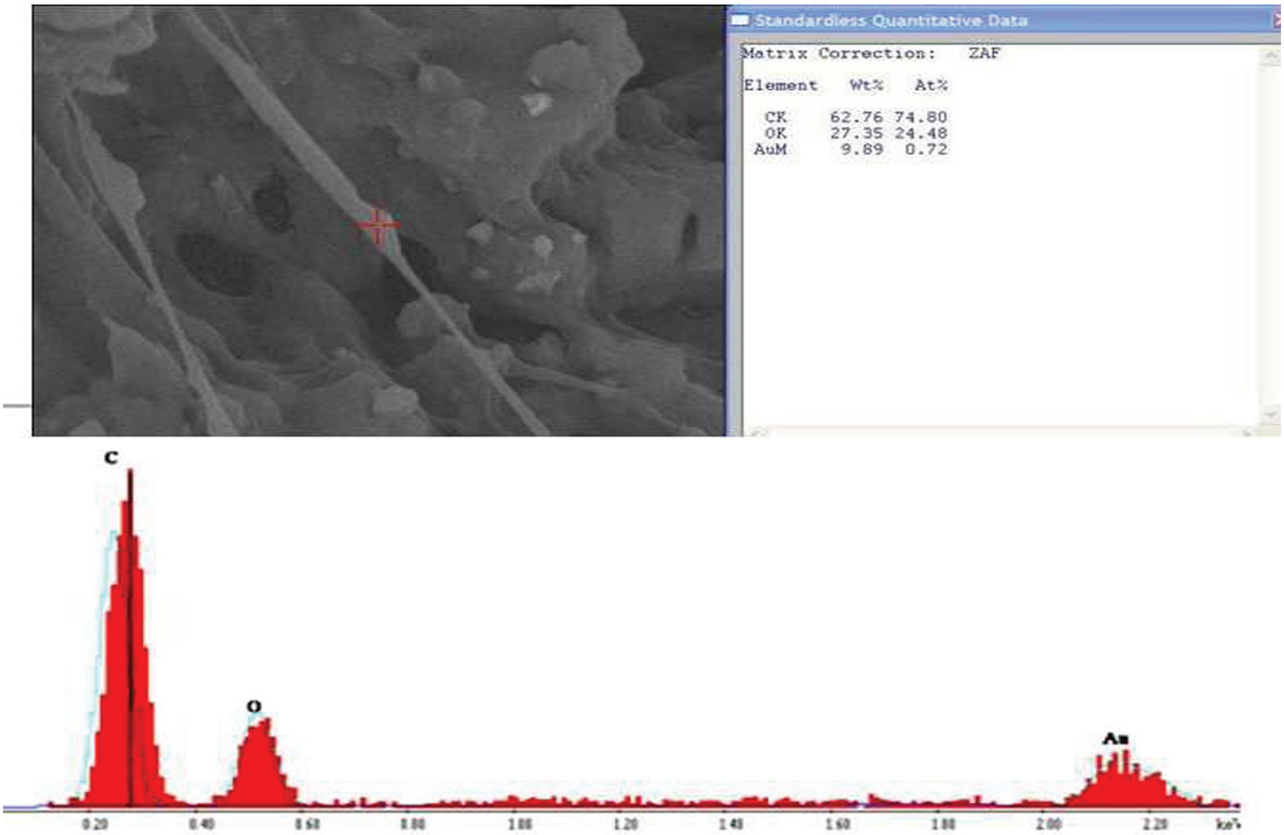


**Fig. 2:** EDX analysis of a stratosphere-filament; note that the filament is collapsed showing it is a tube and not a solid particle.

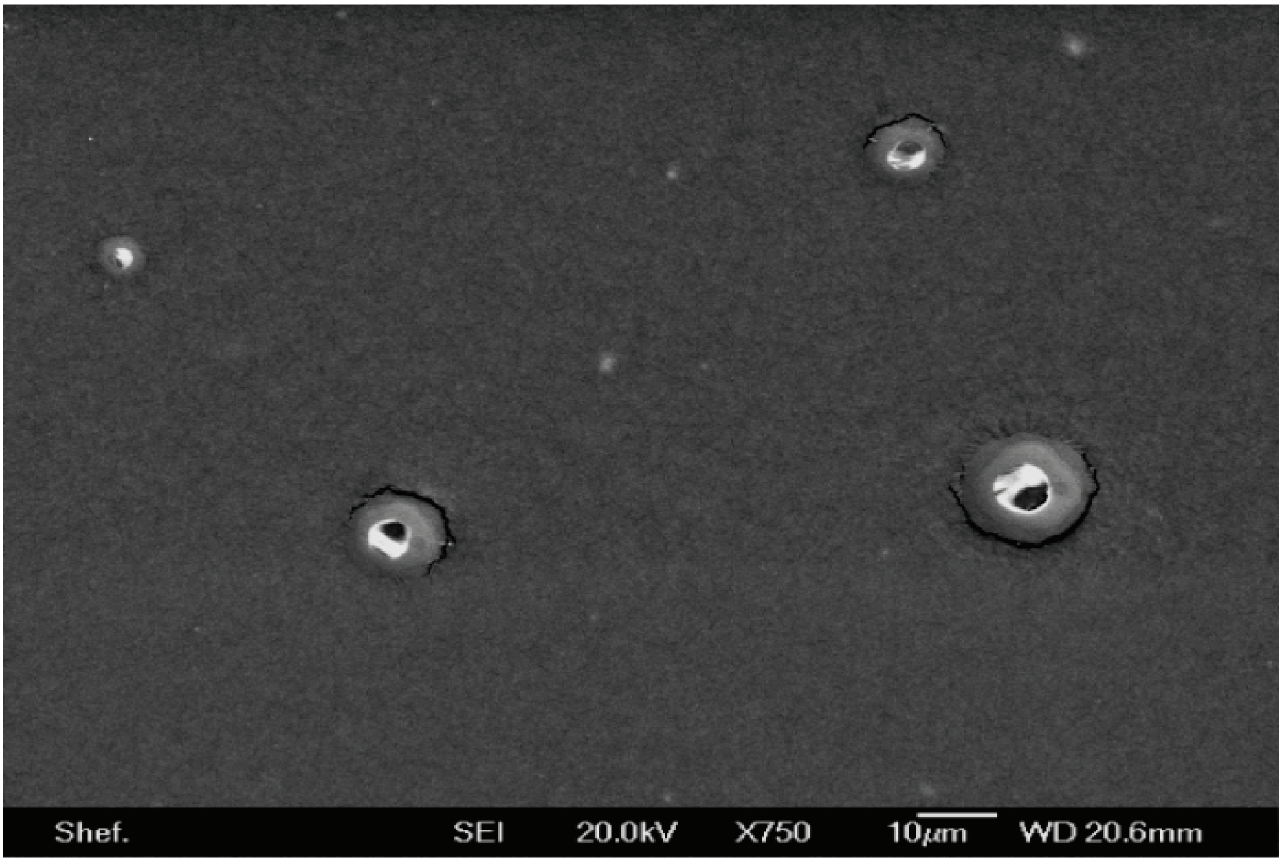


**Fig. 3:** Microcraters discovered on exposed E/M stubs, showing a component of higher speed mineral dust incident from space.

often knee-jerk criticism of contamination was refuted when similar organisms were subsequently isolated by other researchers from the stratosphere (Imshenetsky *et al*., 1978, Yang *et al*., 2009, Smith *et al*., 2010. The fact that these workers employed a variety of sampling procedures and laboratory processing approaches to reach the same conclusion reduces the probability of contamination to the point where we can be confident that viable bacteria exist in the high stratosphere. Hopefully, future sampling trips to the stratosphere by other workers will similarly confirm our current findings that filamentous organisms, like the ones shown here, can be found in this region.

### Micrometeorite craters on exposed E/M stubs

A crucial piece of evidence supporting our conclusion that the particles adhering to the exposed stubs were indeed derived from infalling cometary meteoroids is the discovery of microcraters such as shown in **Fig. 3**.

Meteoroids enter the Earth at high speed (~20–60km/s) and millimetre-sized particles mostly burn up at heights exceeding 80km. Much larger cometary fragments that are part of the meteor stream which encounter air masses comparable to their own mass would be slowed down to speeds below a few km/s and break-up into a shower of smaller particles when they reach heights of < 40km. We would thus expect both high and low speed meteoroids impinging on the stub surfaces. The microcraters seen in **Fig. 3** probably arose from higher speed mineral dust grains of ~2 micron in diameter, producing craters about 5 times as large. These are very similar to craters found on aluminium foil surfaces during the Stardust Spacecraft encounter (January 2004) with dust from comet P/Wildt 2 at a relative speed of 6km/s (Kearsley, *et al*, 2008).

Finally, we have commented at length elsewhere on other reasons why we believe that biological entities present in the stratosphere originate from space rather than Earth (Wainwright *et al*., 2013a, b). The same reasoning allows us to conclude that the filamentous microorganisms shown here is of a non-Earth origin.

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