Ultrastructure and Adhesive Mechanisms of the Biological Spring, 
*Vorticella Convallaria*, Studied Via Atomic Force Microscopy

By

Rafael E. Bras

Submitted to the Department of Materials Science and Engineering 
in Partial Fulfillment of the Requirements for the Degree of

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ABSTRACT

The rod-like, contractile stalk of the single-celled peritrich Vorticella Convallaria can collapse into a tightly coiled helix in less than 1/60 second, a rate ~1000 times its cell body size per second. The contractile stalk (25um-300um in length, ~ 4um diameter) consists of several membranes stiffened by extracellular fibers and helical assembly of larger protein bundles, known as batonets, surrounding an asymmetrically located protein bundle of roughly parallel filaments known as the spasmoneme. The contraction mechanism is thought to be the entropic collapse of spasmin, the negatively charged polyelectrolytic protein that composes the spasmoneme, via Ca$^{2+}$ screening of electrostatic repulsion. The structure and chemical composition of the stalk and spasmoneme are largely unknown. After culturing Vorticella Convallaria, samples were prepared by filtering detached cells. The resulting high cell content aqueous solution was then placed on freshly cleaved mica substrates and allowed to dry for ~30min, until all water had evaporated. The samples were then imaged via contact mode atomic force microscopy (AFM). The structure of stalk was revealed in great detail. The following was clearly visualized; the helicity of the stalk, the spasmonene (3um diameter), batonets (180nm), the outer stalk sheath membrane morphology and folds in the sheath membrane, and organells in the stalk. We were also able to image a circular stalk "foot" (2.8 um diameter) where the organism attached itself to the substrate, as well as what appears to be the biological "glue" used for surface attachment.

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1 Introduction and Background

1.1 Why study *Vorticella Convallaria*?

The ability of biological systems to manipulate structures on the molecular and atomic scale often leads to materials with unusual properties. Through the study of biological materials, it may be possible to develop novel approaches to many materials science problems by mimicking the solutions found in nature. One area that could stand to benefit from the study of biological systems is the development of microactuators. Microactuators have a wide variety of potential applications and could be used in everything from electronics to artificial muscles. Many living cells make use of microactuators to achieve mobility. Examples of such mechanisms include the molecular engines that drive flagella, cilia, and pseudopods. Many microactuators found in nature are based on the same actin-myosin system that is the basis of the human muscle. The fastest and one of the most powerful microactuators, however, is the contractile stalk of the *Vorticella* family of peritrichs [10].

During the sessile stage of its life cycle the *Vorticella* consists of a bell shaped body with a cilia lined oral cavity at one end and a long stalk on the other. The foot of the stalk attaches to a substrate, such as a rock, plant, or even an aquatic animal. The stalks of these organisms are capable of contracting at astounding speeds. *Vorticella* move at such a rate that they would travel fifteen times their own body length in less than a second [11]. W. B. Amos estimated the instantaneous power involved in the contraction to be 500 times the average power of human skeletal muscle [3]. Despite having been first observed by the Dutch scientist Antony van Leeuwenhoek three hundred years ago, the mechanics and the mechanism by which these organisms contract their stalks are not well understood [7, 9]. Studying *Vorticella* could point the way towards the development of novel actuator systems.
The organelle of interest is the contractile stalk, which consists of a series of membranes surrounding the contractile organelle, a rod like structure known as the spasmoneme. The spasmoneme is believed to be composed of the protein spasmin [3]. A few of light and transmission electron microscopy studies have been done concerning the structure of *Vorticella* [1, 2, 4]. Despite these studies, the structure and composition of spasmin is still unknown, though it is clear that Ca$^{2+}$ ions play a major role in the contraction of the spasmoneme [4, 1, 2, 5, 16, 7]. Even less is known about the mechanism by which *Vorticella* adheres to so many different substrates. It is believed that the foot of *Vorticella* stalks secretes a “glue” of some sort, probably a biopolymer. Beyond this guess the mechanism by which *Vorticella* attach to their surroundings is unknown.

The atomic force microscope (AFM) is a powerful and versatile tool with atomic scale resolution and the capability to image in physiological conditions. Atomic force microscopy is based on the principle that the forces between the tip of a sharp, low spring constant cantilever and the atoms of a surface can be measured by monitoring the deflection of the cantilever. By rastering the cantilever across a surface, a very detailed height map can be produced. Hopefully, probing the structure of the *Vorticella* stalk with AFM of will reveal new insights into its properties. This will eventually allow the development of micro actuators based on spasmin or a similar polymer. The “glue” that *Vorticella* use to attach to substrates is also worthy of study, since it is probably a non-toxic water resistant adhesive of considerable strength.

1.2 Anatomy of the *Vorticella Convallaria*

*Vorticella Convallaria* are a species peritrich ciliate commonly found in fresh water ponds and streams. They have a polymorphic life cycle, the majority of which is spent in the
feeding stage as a sessile stalked zooid called a trophant. This organism also assumes a motile form known as a telotroch. The trophant form will metamorphose into a telotroch when exposed to an unfavorable environment or following mitosis (V. Convallaria only share multiple zooids to a stalk during fission). Other minor forms exist as a byproduct of sexual reproduction [8]. During the trophant stage V. Convallaria subsist by sweeping bacteria and other food particles into their mouths with swirling currents created by the rapid beating of peristomial cilia.

The cell body (or zooid) of the trophant is a vase shaped mass, typically ~30µm in length and ~20µm wide, as shown in Figure 1.1. The zooid contains most of a Vorticella’s organelles, such as the food vacuoles, micro and macro nuclei, endoplasmic reticulum, and mitochondria. The anterior region of V. Convallaria consists of an oral region surrounded by cilia. These cilia can be seen in Figure 1.2 as a dark blur around the edge of the mouth. The posterior end of the cell narrows into a small region where a semi-permanent junction connects the long contractile stalk attaches to the zooid. This junction is known as the scopular region. The contractile stalk is about 2.9 µm wide and can be anywhere from 20 µm –300µm long. The other end of the stalk attaches to a substrate, such as rocks, aquatic plants, and even aquatic animals via an adhesive pad [7].
Figure 1.1 Diagram of a *V. Convallaria* trophant.

Figure 1.2 A phase contrast micrograph of a *V. Convallaria* taken during this study by Arpita Upadhyaya.
A few of light, transmission and scanning electron microscopy studies have been done concerning the structure of Vorticella [4, 1, 2, 16, 5, 16, 7]. The organelle of interest is the contractile stalk. The contractile stalk is known to consist of a series of ~7.5nm thick tri-laminar membranes, stiffened by microfibrils of protein known as Batonnets and a fibrillar matrix. A large organelle known as the spasmoneme runs through the stalk inside the central plasma membrane ~2.5 µm in diameter. [4]. The spasmoneme is the organelle responsible for contraction and is believed to be composed of the negatively charged poly-electrolytic protein spasmin. Birefringence measurements [3] and electron microscopy [4, 2, 5] show that when the spasmoneme is contracted it consists of diffuse bundles of weakly crosslinked, roughly parallel

Figure 1.3 Diagram of the anatomy of the contractile stalk of a V. Convallaria.[4]
filaments, each 2-5nm in width. Tubules about 60 nm diameter with membraneous walls and heterogeneous contents run longitudinally among the spasmin filaments and may be calcium. These tubules may be for storing and transporting the calcium needed for contraction. The spasmoneme runs slightly off center in a helical conformation when the stalk is extended, as illustrated in figure 1.3. This helps ensure that the stalk will twist into a coil during contraction. Mitochondria, up to 1.5 µm in diameter, are also found in the stalk. These most likely supply energy to the pumps that must regulate the concentration of the calcium ion that triggers contraction. The specific composition and structure of spasmin remains unknown [4].

The mechanism by which the adhesive pad of V. Convallaria adheres to so many different substrates is unknown. It is believed that the organism secretes a “glue” of some sort, probably a biopolymer.

1.3 Theoretical contractile mechanism

The structure and composition of spasmin is relatively unknown, though it is clear that Ca^{2+} ions play a major role in the contraction of the spasmoneme. Experiments have shown that even after the stalk has been severed from the cell body, it can still be made to contract and extend by increasing then decreasing the calcium concentration in the surrounding media [17, 12]. The same study by Moriyama found that the extension curve of a contracted spasmoneme of an organism related to Vorticella (giant Zoothamnium sp.) was that of a rubbery elastic. Brirefringence experiments also showed the filaments of the spasmoneme to be more strongly aligned in the extended state than the collapsed state [17]. Based on this data it seems likely that spasmin is an electrolytic polymer. The electrostatic repulsion between negative charges along spasmin chains would stiffen and straighten a bundle of these fibers. This corresponds to the
extended state of the stalk. When calcium ions (Ca\(^{2+}\)) are introduced, they would be attracted to the negatively charged regions and shield the repulsion’s between the spasmin fibers. The result is that the spasmin collapses into an entropic rubber as shown in figure 1.4. [10].

Figure 1.4. Diagram illustrating the collapse of negatively charged spasmin fibers in the presence of Ca\(^{2+}\) ions.

1.4 Atomic Force Microscopy

Atomic force microscopy (AFM) is a powerful and versatile tool with atomic scale resolution and the capability to image in physiological conditions. Atomic force microscopy is based on the principle that the forces between the tip of a very tiny cantilever, with a low spring constant, and the atoms of a surface can be measured by monitoring the deflection of the cantilever. By rastering the cantilever across a surface, a very detailed height map can be generated [13]. Current devices use a silica or silicon nitride cantilever controlled through a feedback loop between the piezoelectric actuators that control movement and a laser system that detects deflection of the cantilever. Figure 1.5 shows a schematic of this feedback loop [6].
AFM has a number of features that make it advantageous for imaging cells. First of all, AFM samples require minimal preparation, they do not need to be stained, coated or frozen as with various tunneling electron microscopy (TEM) and scanning electron microscopy (SEM) techniques. Unlike electron microscopes, samples can be imaged in fluid environments, allowing such things as living cells, protein adsorption, and crystal growth to be observed. Even in ambient conditions, a thin layer of water on hydrophilic substrate surfaces, such as mica, keeps the structures partially hydrated with a water layer 0.2-5nm thick, depending on humidity [14]. The high lateral resolution of AFM (less than 1nm in some cases) achieved on biological samples, has revealing detailed information on the conformation, spatial arrangement,
attachment modes, etc. of adsorbed species. Finally, AFM is also capable of a number of techniques complementary to topography mapping, which provide information on other surface properties, such as stiffness, hardness, friction, or elasticity.

Hopefully, probing the structure of the *Vorticella* stalk with AFM of will reveal new insights into its properties. This will eventually allow the development of micro actuators based on spasmin or a similar polymer. The “glue” that *Vorticella* use to attach to substrates is also worthy of study, since it must be a non-toxic water resistant adhesive of considerable strength.
2 Experimental Methods

2.1 Culture media preparation

Cell culture media was prepared using methods modified from those described [15] in “A Novel Method for Mass-Culturing *Vorticella*”, for every liter of solution, two grams of Pines International Inc. powdered wheat grass were mixed into distilled deionized water. This mixture boiled using a hotplate/magnetic stirrer for approximately 5 minutes. The media was then allowed to cool and more water was added to maintain a constant volume. The media was then filtered through a 5µm Nitex® (woven nylon) filter from Sefar America Inc. using a vacuum pump as shown in Figure 2.1. The flask was then autoclaved for twenty minutes following sterilization the media was transferred to smaller sterile containers in order to limit the possibility of contaminating an entire batch of media. Once cool the media, pH 6.09, was ready to use.

![Figure 2.1 Schematic of the lab setup used during the filtration step of media preparation.](image)
2.2 Culturing

The cultures we prepared were derived from a sample of *Vorticella Convallaria* generously provided by Dr. Howard Buhse of the University of Illinois, Chicago. The culturing method used was a modified version of that described by Vacchiano et. al in “A Novel Method for Mass-Culturing Vorticella.” Our organisms were incubated in 500mL and 1L Pyrex erlenmeyer flasks. A high surface area to volume ratio is desirable when culturing *Vorticella* as they require attachment surfaces to feed. Thus only 50mL of media was used for cultures in 500mL flasks and only 100mL in 1L flasks. In order to maintain culture growth, flask media was replaced by pipetting up the old culture media and adding fresh cell culture media diluted fifty percent by volume with spring water. Though some cells are lost in this process, most of the *V. Convallaria* remains attached to the bottom and side of the flask.

It is important to note that fully concentrated media was not used at any time during the culturing process. The number of *Vorticella* found in our cultures decreased markedly if we used fully concentrated media. This may be due to the fact that bacteria are present in the culture as food for the *V. Convallaria*. It may be that the concentrated media presents such a rich environment that bacteria proliferate too quickly and become overabundant, adversely affecting the *V. convallaria*.

In preparation to starting new cultures, culture flasks were refreshed and then shaken on a horizontal shaker overnight, approximately 12 hours, at about 80 rpm. This is done to dislodge *Vorticella* from the flask and promote the formation of telotrochs. The earlier step of rejuvenating the media also promotes telotroch formation. Following shaking the cell enriched media is immediately transferred to a clean sterile flask with a sterilized pipette. This must be
done immediately following removal of the flask from the shaker to avoid the reattachment of *V. Convallaria* to the substrate. The cultures were then allowed to incubate at room temperature. It appears that unless cultures are transferred to clean sterile flasks once every 3-4 weeks cell yields decrease significantly, that is samples had fewer *V. Convallaria* following filtering after several weeks without transfer. Replacing the wheat grass media with inorganic media, 0.24mM potassium chloride (KCl) and 0.24mM magnesium sulfate (MgSO₄) in distilled deionized water, is also advisable on occasion to reinvigorate the cells as this promotes sexual reproduction among the *V. Convallaria* [9].

### 2.3 AFM Sample Preparation

In order to perform AFM imaging it is important to remove as much bacteria as possible from the media. Only cultures at least a week old should be used for sample preparation. While it is possible to image the cells in air despite bacteria, the bacteria are imaged as well and can obstruct features on the *V. Convallaria*. To reduce the amount of bacteria, samples were filtered before being imaged. First, cultures were shaken over night, (ten to twelve) hours, at 80 rpm on the table shaker to detach the cells from the flasks. This results in the formation of telotrochs and unattached trophants. Though it was not necessary for imaging, cultures can be further cleaned by adding another step prior to filtering. After the initial shaking the culture can be transferred to a newly sterilized flask. *Vorticella* attach more quickly to substrates than bacteria and biofilm, so by waiting about eight hours, the time after which more than 55% of the *V. Convallaria* should have reattached to the flask [15], and then refreshing the media with inorganic media, removes much of the bacteria. This cleaned culture can then be shaken at the previous time and speed to
detach *V. Convallaria* for filtering. Obviously, adding this step can substantially reduce the cell yield.

Filtration is achieved by pipetting up the cell enriched media of the shaken flasks and filtering through the apparatus shown in figure 2.2. Two woven nylon filters are used to purify the media. A 35um filter screens out large clumps of bio-film, while bacteria and single *Vorticella* pass through the filter. A 5um filter allows bacteria to pass while *V. Convallaria* are retained. The 5um filter is then inverted and rinsed into a petri dish or onto a sample substrate with a small amount of IM inorganic media. This also concentrates the cells and purifies the culture.

AFM samples were prepared in two ways. One method was to collect droplets of five times concentrated purified cell enriched media directly from the rinsing process, by filtering directly onto a freshly cleaved, pre-mounted muscovite mica substrates (*Structure-Probe, Inc.* grade of...
mica). These sample were then allowed to dry for ~1 hour before imaging. The second method was to collect media (concentrated from 50mL to 1.5mL) into a 5mL petri dish already containing several freshly cleaved and mounted Mica substrates and 1.5mL of IM. These samples were then allowed to incubate for 3-5 hours, providing the Vorticella the opportunity naturally attach to the substrates and to develop stalks. Once the samples were done incubating they were gently removed from the media with tweezers and allowed to dry for one hour before imaging.

These different sample preparation techniques provided different types of images and presented different problems. On several occasions the droplet collection method resulted in the formation of large clumps of cells on the substrate. These high cell density clumps (tens of cells) presented a sample that was too large and confused to image properly with the AFM. However, when the cell concentration was lower and the V. Convallaria more dispersed, these types of samples provided images of cells with long, mature stalks. The natural attachment method ensured that the cells are well distributed over the sample, though there were often samples with few or no cells within a imageable area. Increasing incubation time increases the number of imageable cells, but also the amount of bacteria present in the sample. The incubation method, however, resulted in outstanding images of the stalk foot morphology of the V. Convallaria. The stalks in these images are usually short and underdeveloped as the V. Convallaria have not had sufficient time to grow long stalks.

Samples were imaged using a Nanoscope IIIa-MultiMode™ atomic force microscope (AFM) (Digital Instruments, Santa Barbara, CA). Both a “J” piezoelectric tube scanner (x/y-range=125 µm, z-range=5 µm) and “E” (x/y-range=10 µm, z-range=2.5 µm) piezoelectric tube scanner in contact mode in air. Deflection and height images were simultaneously recorded in
constant force mode using oxide sharpened V-shaped silicon nitride tips mounted on cantilevers at scanning rates of 1-2 Hz and sample rates of 512 samples per line. A variety of cantilevers were used with a nominal spring constant of 0.32 N/m, 0.12 N/m, 0.10 N/m, or 0.06 N/m. The scan angle was set such that the fast scan axis was perpendicular to the stalk. The force was kept at the lowest possible value by continuously adjusting the set point during imaging. Both trace and retrace signals were acquired and compared. Images were flattened and plane-fitted as required. Dimensions were recorded from AFM images at five different locations. Averaged values and standard deviations for these measurements are reported in appendix A.
3 Results and Discussion

3.1 The cell body

The initial goal of this project was to image *V. Convallaria* in their natural environment, liquid water. This proved to be extremely difficult for several reasons. The most significant of these is that it is very difficult to force *V. Convallaria* to lie flat on a surface. These organisms prefer to extend their stalks perpendicular to the surface on which they are attached. This prevents imaging via AFM as the vertical limit of the AFM is only 5 µm. The second problem was finding a way of killing the *Vorticella* without damaging their structures. Live *V. Convallaria* are simply too active to image via AFM and would likely damage the cantilevers with their contractions.

It was possible, however, to image the *V. Convallaria* in air using the protocols described in chapter 2. The image in figure 3.1 is one of the images acquired of an entire *V. Convallaria*. It

![Image of V. Convallaria](image)

**Figure 3.1.** A 72µm x 72µm scan of a *V. Convallaria*. The scan rate was 1.5Hz with a 0.12N/m cantilever and the “J” scanner.
was taken in air using contact mode AFM. In this image the cell body, 48.5±0.1µm in length and 48.5±0.9µm in diameter, is clearly defined and a number of key features are visible. This cell has collapsed as a result of the drying process. A number of large voids 6.1±1.3µm At the top of the cell body the oral cilia can be seen. It should noted that their was quite a bit ov variation in cell size from organism to organism. This cell is also more circular than bell shaped, suggesting that this *V. Convallaria* contracted as a response to the harsh environment of a drying substrate. Near the bottom of the image a short stalk, about 12µm in length, can be seen extending from the cell body and attaching to the substrate. The short length of the stalk may seem a little surprising. This is due to the fact that this sample was prepared using the natural attachment sample preparation method described in chapter 2, which allows the cells to incubate and attach to the substrate over several hours. The cell shown here simply did not have sufficient time to grow a longer stalk.

### 3.2 Features of the Stalk

Imaging of *V. Convallaria* stalks in air was also successful. A number of key features, such as the various stalk membranes, the spasmoneme, and the batonnets were visualized with the AFM. The 3nm fibrils that compose the fibrilar matrix were not observed in any of the AFM images. Figures 3.2 and 3.3 are deflection images of extended *V. Convallaria* Stalks. Figure 3.2 is a higher resolution 2D deflection image in which the batonnets are clearly visible. To the left and right of the stalk a number of oddly shaped bumps are visible on the substrate. This debris is particles of biofilm that were not screened out during the filtration process. It is very difficult to completely remove all debris from samples, as clumps of biofilm can be attached to the adhesive
Figure 3.2 A 8µm x 8µm scan size, deflection image of a *V. Convallaria* taken with a 0.12 N/m cantilever and the “J” scanner.

Figure 3.3 A 6µm x 6µm scan size, deflection image of a *V. Convallaria* stalk. The scan rate was 2.03Hz a 0.12N/m cantilever and the “J” scanner.
foot of a *Convallaria* that has been shaken off its flask. In figure 3.3 the central the cytoplasmic tube is clearly visualized draped over the spasmoneme.

Both the batonnets in figure 3.2 and the membrane draped spasmoneme in figure 3.3 are more clearly visualized in by the 3-D height images shown in figures 3.4 and 3.5. By converting the height image from the AFM scan into a 3D rendered surface we can more easily see folds and wrinkles in the membranes as well as the shape of the spasmoneme underneath the inner plasma membrane. In the 3D image the rounded protrusions next to the spasmoneme underneath the plasma membrane are more obvious. These protrusions are probably mitochondria. In many TEM images of *Vorticella* the cytoplasm of the tube is not well preserved and the mitochondria are not visible. Image processing was done using WSxM, a scanning probe microscope.

Figure 3.4 The height image of the stalk in figure 3.3 converted to a 3D rendered surface.
acquisition and image analysis freeware program designed and distributed by Nanotec Electronica of Spain.

From the height data recorded by the AFM, measurement of various features were taken using horizontal line profiles. Dimensions were recorded from AFM images at five different locations. Averaged values and standard deviations for these measurements are summarized in the appendix. Height measurements are demonstrated in figure 3.7, which is a horizontal line profile along the dark line in figure 3.6, the height image of the stalk from figure 3.3. Steps in the line profile distinguish the various features. The spasmoneme was found to be $0.8 \pm 0.1 \mu m$ in diameter, while the cytoplasmic tube was $1.6 \pm 0.2 \mu m$ in diameter with an entire stalk diameter of $4.5 \pm 0.1 \mu m$. The outer sheath membrane was found to be $61 \text{nm}$ thick. The batonnets were found to be $\sim 0.2 \pm 0.05 \mu m$ in diameter.
**Figure 3.6** A 6µm x 6µm scan size, height image of a *V. Convallaria* stalk. The scan rate was 2.03Hz using a 0.12N/m cantilever and the “J” scanner. This height image is of the same section of stalk as figure 3.3.

**Figure 3.7** The horizontal line profile taken along the dark line in figure 3.6.
Images of both extended and coiled stalks were acquired using the AFM. In TEM studies all the stalks imaged are coiled as a result of the fixation process. Shown in Figure 3.8 is the coiled stalk of a *Vorticella*. This image displays a 2-D deflection AFM image of 5 left-handed rotations of a helically coiled stalk. The stalk has a diameter of 4.2±0.5 µm and a maximum height of 0.76.0±0.1 µm. The helix has a diameter of 9.8±1. µm and a pitch of 4.7±0.9 µm. Pitch is defined as the distance along the helical axis that it takes for the stalk to rotate by one full turn. The batonnets, 0.27±0.06µm in diameter, can also be seen following the coils of the stalk. In some places the batonnets can be clearly seen to stiffen the plasma membrane, which was 0.16±0.05 µm thick, causing discrete bends in the outer sheath rather than a smooth coils as one might expect. This agrees with the current theory that the role of the batonnets is to asymmetrically stiffen the stalk so that it will coil upon the contraction of the spasmoneme. In this image the cytoplasmic tube has diameter of 3.0±0.3 µm and appears to be partially hydrated, as it has not collapsed to reveal the spasmoneme and mitochondria.

In some cases the inner plasma membrane of the stalk had degraded allowing the spasmoneme and degraded mitochondria to be imaged. The image in figure 3.9 is an example of this. In this image the stalk diameter is 4.2±0.6 µm and total stalk length is 73.3±0.5 µm, with a maximum height of 0.2±0.04 µm. In this image the degraded mitochondria can be seen forming clumps all along the spasmoneme. The spasmoneme has a diameter of 1.5±0.3µm and a pitch of 10.9±1.8 µm. The smaller distinct granular structures, assumed to me mitochondria, are 0.9±0.2 µm in size. The batonnets in this image traverse the stalk on a helical path opposite to that of the spasmoneme and are discontinuously staggered. Each has a diameter of 0.28±0.05µm and length 6.3±1.3µm. The outer sheath membrane was found to have a thickness of 22±4 nm. Thus it would seem that there is little cellular material left outside the cytoplasmic tube in this
sample. Two long round features can be seen extending off of the viewed area along the sides of the stalk. These features are actually bacteria that have contaminated the sample. Fortunately, they did not interfere with the imaging of the *V. Convallaria* stalk in this case. These bacteria tend to form long chains, which can become entangled with the *V. Convallaria* and are difficult to wash away during the filtration process.

Comparison to SEM and TEM shows that AFM imaging gives feature sizes that are consistent, but not the same. The spasmoneme was measured to be 1.2µm in diameter [Amos 1972] in TEM studies spasmoneme widths found by AFM are both above and bellow this value. This could be due to the differences in preparation methods or variation among individual organisms. AFM values for the stalk diameter are generally larger than those reported in TEM studies, which report stalk diameters of 2.9µm [4]. This discrepancy is probably due to the collapse of the stalk, causing it to spread out and appear to have a larger diameter.
Figure 3.8 A 35µm x 35µm deflection image of a coiled fragment of *V. Convallaria* stalk. The scan rate was 1Hz with a 0.32 N/m cantilever and the “J” scanner.

Figure 3.9 A 30µm x 30µm deflection image of an extended *V. Convallaria* stalk. The scan rate was 1Hz with a 0.1 N/m cantilever and the “J” scanner.
3.3 Batonnet Morphology

One of the most prominent features of the *Vorticella* stalk are the batonnets. As stated earlier, the main purpose of these fibrils is to ensure that the contraction of the spasmoneme results in the coiling of the entire stalk. With a larger scan area, the battonets can be seen forming a coil with the opposite handedness of the extended spasmoneme, as expected. Examining the batonnets, zooming in on the boxed region in figure 3.10, more closely reveals that these structures do have a substructure consisting of 2-3 subfibers 53±15nm in diameter. A horizontal line profile along the grey line in the magnified image gives the profile shown in figure 3.11. This confirms TEM observations that the batonnets are composed of subfibers, but disagrees as to the number and size of these fibers. Literature suggests that the batonnets are composed of subfibers 15-3nm in diameter [4]. This could be explained by an intermediate structure. The fundamental protein filaments of the batonnet could be 15-3nm in diameter strands that form intermediate bundles that are ~60nm in diameter. These fibers might then bundle to form the large 300nm wide 6.3±1.3µm long fibrils that are the batonnets.
**Figure 3.10** (left) A 10µm x 10µm deflection image of an extended *V. Convallaria* stalk. The scan rate was 1.5Hz with a 0.32N/m cantilever and the “J” scanner. (right) An expanded view of the boxed region in the image on the left. Batonnetsubfibers are clearly visible.

**Figure 3.11** The horizontal line profile along the grey line in figure 3.10. The batonnets can clearly be seen to separate into subfibers 53± 15 nm in diameter.
3.4 Foot Morphology

By allowing the *V. Convallaria* to incubate in inorganic media with mica substrates it was possible to image the morphology of the attachment site of the *Convallaria* to the substrate. These images revealed the morphology of the *Vorticella* foot, a part of the organism that has not previously been examined in detail. As expected, the plasma membrane, diameter of ~4.5±0.1 µm and thickness~23±2nm, cytoplasmic tube, diameter of ~1.6±0.2 µm, and the spasmoneme, diameter of ~0.8±0.1µm, are all visible at the foot of the *Vorticella*. A circular ridge defines the foot region. Horizontal line profiles, see figures 3.15 and 3.17, of the foot measure the diameter of the foot to be 3.3±0.1µm in diameter with a height of 0.2±0.06µm.

The most interesting feature, however, is a large roughened area that surrounds the foot and is shown in figures 3.12 through 3.14. The roughened region in these images is 14.1±0.3µm in diameter. This region may be coated with the biological adhesive that the *V. Convallaria* use to attach to substrates. Figure 3.15 is a 3D rendered image of figure 3.16 that helps to visualize the morphology of the foot region. These observations were reproducible, that is we were able to observe this region of increased roughness surrounding the foot of the *Vorticella* in several different samples. The observation of this region highlights the utility of the AFM, it would have been very difficult to observe this result with TEM or SEM. Also, the effect of various preservation treatments on the adhesive produced by *V. Convallaria* is unknown.
Figure 3.12 A 32µm x 32µm deflection image of an extended *V. Convallaria* stalk. The scan rate was 1.5Hz with a 0.32N/m cantilever and the “J” scanner.

Figure 3.13 A 15µm x 15µm deflection image of an extended *V. Convallaria* stalk. The scan rate was 1.5Hz with a 0.32N/m cantilever and the “J” scanner.
Figure 3.14 A 9µm x 9µm deflection image of an extended *V. Convallaria* stalk. The scan rate was 1.5Hz with a 0.32N/m cantilever and the “E” scanner.

Figure 3.15 A 3-D height map of the image in figure 3.16 highlighting the morphology of the foot region.
Figure 3.16 A 5µm x 5µm height image of an extended V. Convallaria stalk. The scan rate was 1.5Hz with a 0.32N/m cantilever and the “E” scanner.

Figure 3.17 The horizontal line profile taken along the grey line in figure 3.17.
4 Conclusions

Atomic Force Microscopy was successfully used to image a number of structures and features of Vorticella Convallaria. This was the first time that Atomic Force Microscopy (AFM) was used to investigate the *V. Convallaria* structure and surface adhesion mechanisms. The helicity of the stalk, the spasmoneme (1.3-2.4 μm diameter), bâtonnets (230-300nm), the outer stalk membrane morphology, and mitochondria in the stalk were all clearly visualized and identified. This data agrees with literature for the most part, observed features are of similar dimensions. The AFM data does tend to indicate slightly smaller feature dimensions. This could be the result of variation between organisms of an artifact related to the sample preparation methods in various microscopy techniques.

The circular adhesive pad (2.8 μm diameter) where the organism attached itself to substrates was also imaged. A roughened region ~14μm in diameter encircling the attachment site was also imaged. This region may be the biological "glue" used for surface attachment and was observed in several different samples.

In several samples it was not possible to image the *spasmoneme* and mitochondria through the cytoplasmic tube. Thus it appears that the stalk can remain partially hydrated for hours after drying or that the cytoplasmic tube is quite stiff.

This work has formed the basis for further exploration of *Vorticella Convallaria*. The next logical step is to continue work on imaging these organisms in liquid environments. This would likely reveal a great deal about the structure of fully hydrated *V. Convallaria* stalks and provide information on the mechanical properties of these the stalk. Further exploration of the roughened region around the *V. Convallaria* foot is also in order. Adhesion measurements through force curves on this region could provide insight into the details of the mechanism by
which V. Convallaria attaches to objects. Some chemical analysis techniques could also be applied to this region in an attempt to identify the composition of the substance surrounding the stalk.
## Appendix A

<table>
<thead>
<tr>
<th>Structure</th>
<th>Figure</th>
<th>Mean</th>
<th>STDEV</th>
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</thead>
<tbody>
<tr>
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6 REFERENCES


