Multiscale approach reveals that *Cloudina* aggregates are detritus and not in situ reef constructions

Akshay Mehра*1,2 and Adam Malоof*

*Department of Geosciences, Princeton University, Princeton, NJ 08544

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The earliest metazoans capable of biomineralization appeared during the late Ediacaran Period (635–541 Ma) in strata associated with shallow water microbial reefs. It has been suggested that some Ediacaran microbial reefs were dominated (and possibly built) by an abundant and globally distributed tubular organism known as *Cloudina*. If true, this interpretation implies that metazoan framework reef building—a complex behavior that is responsible for some of the largest bioconstructions and most diverse environments in modern oceans—emerged much earlier than previously thought. Here, we present 3D reconstructions of *Cloudina* aggregates, produced using an automated serial grinding and imaging system coupled with a recently developed neural network image classifier. Our reconstructions show that *Cloudina* aggregates are composed of transported remains while detailed field observations demonstrate that the studied reef outcrops contain only detrital *Cloudina* builds, suggesting that *Cloudina* played a minor role in Ediacaran reef systems. These techniques have wide applicability to problems that require 3D reconstructions where physical separation is impossible and a lack of density contrast precludes tomographic imaging techniques.

Ediacaran | reefs | early life | three-dimensional reconstructions | biomineralization

Although the Ediacara biota largely are preserved as soft-bodied impressions in fine-grained sediment (1), there are several biomineralizing metazoans within Ediacaran strata (2), chief among them the millimeter-to-centimeter–scale index fossil, *Cloudina*. *Cloudina* has been described as a gregarious, epibenthic, sessile suspension feeder (3, 4) that attached to microbial substrates through an apical end (5, 6) and that responded to environmental changes by altering its growth strategy (7). Researchers have suggested that *Cloudina* individuals, when found in aggregated clusters, exhibit preferred horizontal-to-subhorizontal–oriented growth (6). On the basis of field observations, workers have proposed that *Cloudina* may have produced calcified framework reefs some 20 Ma before the advent of the archaeocyathan builds of the Lower Cambrian (521 Ma, ref. 8), which are widely regarded to be the first metazoan-mediated reefs. In modern oceans, reefs built by metazoans comprise laterally extensive, organically formed wave-resistant structures that alter surrounding environments and contain high species biodiversity. Constraining the timing of the first metazoan-mediated reefs can provide insight into how (and perhaps why) the act of reef building emerged; the environmental conditions that cultivated such behavior; the evolutionary journey that led to both biomineralization and the construction of frameworks; and the effect such reefs had on ecology, evolution, sedimentology, and platform architecture.

To determine whether *Cloudina* built reefs, in situ 3D information about the structure and orientation of individuals within aggregates is required. Rarely, *Cloudina* specimens are silicified or phosphatized and individual specimens can be isolated from the host rock for study via acid dissolution (9–11). However, most *Cloudina* remains are calcified and have similar physical properties to those of the surrounding limestone or dolomite matrix, precluding physical separation or the use of traditional computed tomography (CT) techniques. The inability to produce in situ 3D reconstructions has led researchers to make measurements of *Cloudina* individuals and aggregates on polished slabs, thin sections, and bedding planes (3, 4, 7). Unfortunately, as noted by previous researchers, 3D spatial and size distributions cannot be estimated from 2D cross-sections (12). Furthermore, synthetic experiments reveal that, in the case of tubular structures such as *Cloudina*, it is not possible to correctly infer orientation from 2D cross-sections (Fig. 1 A–C), and diameter measurements made on cross-sections through curved and/or elliptical tubes are subject to a large degree of error (as great as 35%; Fig. 1 D and E). To address these issues, we serially ground, imaged, and traced *Cloudina* fossils using the Grinding Imaging Reconstruction Instrument (GIRI) at Princeton University (see Materials and Methods for discussion).

Georeferenced observations, along with physical samples, were collected from three Ediacaran *Cloudina*-bearing reef systems: the pinnacle reef complex at Driedoorvlakte Farm near Rehoboth, Namibia (Figs. S1 and S2B); the patch and sheet microbial builds at Zebra River Farm south of Naukluft, Namibia (Figs. S2B and S3); and recently exposed stromatolite bioherms on Salient Mountain in Canada (Figs. S2A, S4, and S5). Each outcrop was subject to a multiscale approach consisting of (i) kilometer-scale drone-assisted mapping to evaluate the extent of the reef; (ii) meter-scale, differential GPS-constrained stratigraphic and

Significance

Little is known about how the Ediacaran index fossil *Cloudina* lived and what impact it had on its surroundings. This uncertainty is due to the fact that *Cloudina* often is preserved with the same mineralogy as the rocks in which it is found; the lack of density contrast means that traditional imaging techniques cannot be used to reconstruct and measure in situ *Cloudina* populations. Recently, researchers have suggested that *Cloudina* was a framework reef builder that actively adapted to changing environmental conditions. In this paper, we use a serial grinding and imaging technique to produce 3D models of *Cloudina* aggregates. Along with detailed field observations, we demonstrate that *Cloudina* populations are detritus and not in situ growth.

Author contributions: A. Mehra and A. Maloof designed research; A. Mehra performed research; A. Mehra and A. Maloof analyzed data; and A. Mehra and A. Maloof wrote the paper.

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Data deposition: The measurement data and the computational source code reported in this paper have been deposited in https://github.com/giriprinceton/cloudina. Due to size constraints, all original raw image data will be provided on request by emailing akmehra@princeton.edu.

1To whom correspondence should be addressed. Email: akmehra@princeton.edu.

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sedimentological observations to identify where Cloudina can be found; (iii) centimeter-scale petrographic and stable isotope analysis to contextualize depositional and diagenetic histories; and (iv) submillimeter-scale 3D reconstructions of in situ Cloudina populations to quantitatively describe the aggregates.

We find that Cloudina specimens primarily are preserved as detrital assemblages consisting of transported individuals within the fill between microbial buildups; that Cloudina thicket, when found, makes up 3% of the host reef; and that Cloudina was easily deformed and may have been composed of a weakly or nonbiomineralized shell. We conclude that Cloudina likely was a minor component of microbial reefs during the Ediacaran and suggest that Cloudina populations may have had an analogous habit to that of present-day serpulid worm buildups, which exist in a wide range of modern environments but are distinct from large-scale, wave-resistant, structural framework constructions.

Field Observations and Reconstruction Results

In Namibia, the Driedoornvlakte and Zebra River Farm reefs represent Neoproterozoic age Nama Group sediments that were deposited on a carbonate ramp in the northern Zaris subbasin following the Damara and Gariep orogenies (13, 14). The Kuibus subgroup, which comprises the lowest Nama and contains Cloudina-bearing sediments, has a maximum age of 620–590 Ma (13) and a minimum age, on the basis of a uranium–lead zircon date in the overlying Schwarzrand subgroup, of 545.1 ± 1 Ma (15). An additional geochronological constraint is provided by an ash bed 270 m above the base of the Kuibus at Zebra River Farm, located in an interbedded microbialite succession above Cloudina-bearing thrombolites (Fig. S2D), which has a reported age of 548.8 ± 1 Ma revised to 547.32 ± 0.31 Ma (15, 16).

The reef at Driedoornvlakte Farm is a 500-m-thick, 10-km-long outcropping that dips ~25°–40° to the south and that, on the basis of changes in reef geometry, has been divided into three distinct units (17). The third—and youngest—unit, which was deposited in an environment of increasing accommodation before drowning by the Urikos shales (17), has been the focus of multiple studies (17–19), including several that proposed evidence for in situ Cloudina growth (4, 6, 7). The third unit contains pinnacle reefs made up of coalescing decimeter- to-meter–scale microbial mounds. The mounds are composed of both columnar or encrusting stromatolites and columnar or massive thrombolites. Fill between the stromatolites and thrombolites, when present, often contains the skeletal remains of Cloudina and the gobelet-shaped biomineralizer Namacalathus (20). Remains of both organisms, along with the modular metazoan Namapoikia (21), also can be found within neptunian dikes distributed throughout the reef. On the fore slope of the reef are meter-scale dolomitized clinoformal grainstones consisting solely of the skeletal remains of Namacalathus and Cloudina. Cloudina thicket, a dense aggregate of individuals without evidence of internal microbial fabrics that has been suggested to represent framework reef building (4, 6), is present in several mounds within the reef. A detailed survey of the reef reveals that Cloudina thicket is sparsely distributed within the outcrop area. Although ~52% of all surveyed points exhibit some form of microbial texture, only ~14% have any sort of skeletal remains present, and fewer than 3% have been identified as Cloudina thicket (Fig. 2).

Two samples of thicket from Driedoornvlakte (named thicket A and thicket B) consist of Cloudina tubes surrounded by a fine-grained, dark micrite matrix that lacks any apparent microbial
remains and surrounding matrix are partially dolomitized, while only the matrix is partially dolomitized in thicket B. Cloudina tubes in thicket A plunge 21.5° ± 10.4° relative to the bedding plane (Table 1) and, while usually trending between 25° and 240°, the specimens do not exhibit a dominant trend (Fig. 4E). The distribution of trend and plunge measurements can be described as variable lineations on a single best-fit plane with a strike of 055.2° and a dip of 22.3° relative to the bedding plane (Fig. 4E). In thicket B, Cloudina individuals plunge 35.8° ± 18.9° relative to the bedding plane (Table 1) and do not exhibit a dominant trend (Fig. 4F).

The outcrop at Zebra River Farm, located up-ramp of Driedoornvlakte, is composed of near-horizontal (1°–2° dip to the northwest) strata that are exposed in a system of river canyons (22). The stratigraphic succession overlies the Kaines quartzite at the bottom of the canyons and consists of alternating fine- to coarse-grained grainstones that are twice interrupted by meter-scale, often dolomitized, microbial reef buildups, which in turn are overlain by a sequence of interbedded microbialites and shales (Fig. S2D). The lower reef occurrence appears as isolated mounding biostromes with poor fabric retention while the upper reef is sheetlike and forms a resistant bedding plane that exposes mounds of thrombolites and associated stromatolites. The mounds exhibit directional elongation and contain skeletal material within the fill between individual microbial buildups. No Cloudina thickest were identified within the Zebra River stratigraphy.

Fill between two thrombolite mounds located in the upper reef unit at Zebra River Farm (Fig. S2D) includes both Cloudina and Namacalathus preserved as dark, recrystallized molds in a light, extremely fine-grained micrite matrix (Fig. 3F). Cloudina can be divided into smaller (outer semimajor axis size <1.25 mm) and larger (outer semimajor axis size >3 mm) size fractions (Figs. 3F and 4G and Movie S2), exhibit elliptical cross-sections, and have no appreciable change in semimajor axis size (Table 1). Internal cavities, especially in smaller Cloudina specimens, are not always distinguishable. When present, interiors are hollow, smooth walled, and filled with micrite. Larger Cloudina have smooth external surfaces while smaller tubes can be either ribbed or smooth. Cloudina plunge 31.6° ± 17.5° relative to the bedding plane (Table 1), and individuals have no predominant trend (Fig. 4G).

In Canada, the isolated biohermal reefs on Salient Mountain outcrop as glacially polished exposures that dip approximately 18° to the southwest on a series of southeast-to-northwest–trending normal fault blocks (Figs. S4 and S5). Each block exhibits variable exposure of the same stratigraphic succession. A series of alternating sandstones and shales transition into an oncrite and stromatolite horizon with no visible skeletal material. Above the horizon is a unit of interbedded shale and massive sandstone that is capped by meter-scale, elongated pink Platella stromatolite bioherms with skeletal material (including Cloudina) in fill. The Platella bioherms are overlain by an upper sandstone succession, which contains two thin dolomitized horizons, both with evidence of poorly preserved stromatolites (Fig. S2C). Although the Platella fabrics (Fig. 3 D and E and Movie S1). Individual specimens have smooth or slightly corrugated exterior surfaces and smooth-walled, hollow interiors that are filled with either micrite or blocky calcite. Cloudina regularly bend, have elliptical cross-sections, and have a consistent semimajor axis size along the length of the tube (Table 1). In thicket A, both the Cloudina remains and surrounding matrix are partially dolomitized.
Fig. 3. Neural network image segmentation technique, sample images, and 3D reconstructions. (A–J) White bars in lower left represent 0.5 cm. T.A., T.B., Z.R., and S.F. refer to thicket A, thicket B, Zebra River fill, and Salient Mountain fill, respectively. (A) Superpixel oversegmentation. (B) Likelihood image illustrating the network-calculated probability of superpixels belonging to a given class (in this case, fossil). (C) Outlines of thresholded superpixels bounding fossils. (D) Slice of thicket A from Driedoornvlakte: 1 depicts dolomitization present throughout the sample while 2 illustrates blocky calcite fill. (E) Slice of thicket B from Driedoornvlakte. (F) Slice of fill from Zebra River Farm. (G) Slice of fill from Salient Mountain: 1 shows a truncated shell. (H) Side view of 3D reconstruction of Cloudina from Zebra River fill. The red dashed lines highlight several “larger” tubes while the yellow dashed lines highlight the more numerous “smaller” tubes. (I) Comparison demonstrating how a single cross-section can appear to be the basal end of a tube (Left) but instead turn out to be a fragmented, open cup (Right). (J) Reconstruction of a single Cloudina tube with flared rims from Salient Mountain.
bioherms are laterally well exposed, no evidence of Cloudina thicket was found.

A sample from the Platella bioherms contains Cloudina and Namacalathus remains that are found within pockets of fill between microbial buildups (Fig. 3G and Movie S3). Skeletal remains are fragmented, incomplete, and, in certain cases, appear to be truncated by overlying microbial layers (Fig. 3G). Internal tube cavities are smooth walled, hollow, and filled with a combination of detrital skeletal material, pink micrite, and submillimeter quartz grains. Cloudina individuals appear to be composed of cemented, connected funnels with flared rims (Fig. 3I and J). Tubes have elliptical cross-sections and exhibit a consistent semimajor axis size along the length of the tube (Table 1). Several (n = 3) 1-mm diameter spheres, with zero to two apertures, also were identified during reconstruction. Cloudina plunge 45.4° ± 19.0° relative to paleohorizontal (Table 1). Trend and plunge measurements are widely distributed, and Cloudina individuals can be described as broadly plunging to the northeast (Fig. 4H).

**Table 1. Aggregated observations from 3D reconstructions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Thicket A</th>
<th>Thicket B</th>
<th>Thrombolite fill</th>
<th>Stromatolite fill</th>
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</thead>
<tbody>
<tr>
<td>Location</td>
<td>Driedoornvlakte Farm</td>
<td>Driedoornvlakte Farm</td>
<td>Zebra River Farm, upper reef unit</td>
<td>Salient Mountain</td>
</tr>
<tr>
<td>Count</td>
<td>29</td>
<td>24</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>Semimajor axis, mm</td>
<td>3.5/4.4/5.0</td>
<td>3.8/4.5/0.0</td>
<td>1.1/1.2/2.3</td>
<td>2.3/3.2/4.3</td>
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<tr>
<td>Semiminor axis, mm</td>
<td>2.7/3.3/3.6</td>
<td>3.2/3.7/4.2</td>
<td>0.9/1.0/1.8</td>
<td>1.9/2.8/3.7</td>
</tr>
<tr>
<td>Length, mm</td>
<td>12.4/15.2/19.8</td>
<td>11.1/16.7/25.4</td>
<td>6.4/8.4/10.6</td>
<td>3.5/3.7/4</td>
</tr>
<tr>
<td>Cross-sectional eccentricity</td>
<td>0.73/0.78/0.83</td>
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<td>0.77/0.85/0.91</td>
<td>0.77/0.82/0.87</td>
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<tr>
<td>Mean change along tube length, %</td>
<td>6.0 ± 4.2</td>
<td>6.4 ± 4.2</td>
<td>9.4 ± 8.2</td>
<td>8.2 ± 6.4</td>
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<tr>
<td>Mean plunge, °</td>
<td>21.5 ± 10.4</td>
<td>35.8 ± 18.9</td>
<td>31.6 ± 17.5</td>
<td>45.4 ± 19.0</td>
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<td>Micrite</td>
<td>Micrite and shell fragments</td>
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<tr>
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<td>None</td>
<td>Matrix</td>
</tr>
<tr>
<td>Flared rims</td>
<td>False</td>
<td>False</td>
<td>False</td>
<td>True</td>
</tr>
</tbody>
</table>

Fields with values separated by shills represent the 25th/50th/75th percentile values.

*Percentage of change of semimajor axis size along tube length.

The absence of apical ends—which, as previously noted, can be easily misidentified in 2D cross-section (Fig. 1E)—and lack of attachment to microbial surfaces suggest that the Cloudina are not growing where they are found, while the semimajor axis size measurements imply that individual tubes represent incomplete segments of whole Cloudina organisms. As a result, the samples are likely detrital assemblages, the same as previously described Cloudina aggregates in Spain, China, Paraguay, Nevada, and Brazil (5, 10, 33, 34).

It could be argued that Cloudina aggregates represent the broken remains of an in situ reef inhabitant. Although the possibility that Cloudina may have been a reef inhabitant cannot be ruled out, the absence of Cloudina thicket in all three study areas suggests that it was, at most, a minor metazoan component of Ediacaran reef systems. Furthermore, distributions of 3D individual tube orientation (Fig. 4 E–G) suggest that the studied assemblages probably were transported before deposition. If Cloudina took advantage of nutrient flux (whether controlled by current or a steady rain of organic matter from above), as suggested by previous researchers (6), individual tubes of an in situ framework would all be oriented in a single, predominant direction (4) with similar plunge angles. In contrast, the trends and plunges of Cloudina in all four reconstructions can be explained by hydraulic processes. In thicket A, individual tubes have similar inclinations relative to the bedding plane but are not pointing in one direction, which can be explained by reorientation by a unidirectional current following deposition at the edge of a microbial buildup. In contrast, Cloudina individuals from thicket B, along with those from Zebra River, exhibit a lack of coherence in both plunge and trend, suggesting rapid settling following transport (35). Finally, at Salient Mountain, specimens do broadly point in one direction but have a range of plunges, suggesting reworking by a turbulent unidirectional or tidal current. Although it is rare to transport shell assemblages over large distances, habitat-adjacent movement of death assemblages in modern environments is a common occurrence (35), suggesting that Cloudina aggregates, when found in Ediacaran reef systems, may be proximally sourced. Deposition following transportation likely produced detrital assemblages with high porosity, which would explain the presence of marine cements as described by previous workers (4, 36).
Fig. 4. Data from 3D reconstructions. (A) Comparison of reported *Cloudina* diameters, including measured semimajor and semiminor axis size from this study. (B) Plot of eccentricities of *Cloudina* individuals from all three samples. Median, maximum, and minimum measurements refer to measurements within each individual. (C) Plot of aspect ratios, comparing tube length to outer diameter semimajor axis measurements. (D) Distribution of minor axis plunge angles from all three individuals. $\mu$ (mean) and $\sigma$ (SD) refer to values obtained by fitting a normal distribution to the data. Dashed lines with labels refer to expected distributions for different compaction scenarios. D, Inset illustrates length and semimajor and semiminor axes on an idealized tube. (E–H) Directional and semimajor axis size distributions of thicket A, thicket B, Zebra River Fill, and Salient Mountain Fill, respectively. E–H, Top show stereonets depicting the mean plunge and trend vector of each specimen with $\alpha_{95}$ confidence limits; unique color and symbol combinations represent individual tubes, some of which bend, thereby yielding larger $\alpha_{95}$ confidence limits. E–H, Bottom show histograms of median outer diameter semimajor axis size.
Reconstructed Cloudina individuals do not have circular cross-sections (Fig. 4D) as previously assumed by researchers when making 2D diameter measurements. If the wide range of Cloudina aspect ratios were the result of compaction, the semiminor axis of individual tubes would be oriented subvertically to vertically (i.e., semiminor plunge angles would be distributed about or near 90°). However, all four samples have normally distributed semiminor axis plunge angles, with means ranging from 24.0° to 42.7° (1σ ranging from 15.3° to 22.7°: Fig. 4D). As a result, it is likely that Cloudina individuals were deformed before burial compaction, during either transportation or deposition. The fact that individual tubes could be so easily deformed suggests that Cloudina was weakly biomineralized. Intrasample measurements reveal that Cloudina shells and the surrounding matrix have similar mean δ¹³C values (Fig. S6). The δ¹³C of shells is controlled by a combination of shell type, primary mineralogy (i.e., whether aragonite or calcite is precipitated), and/or diagenesis (37). Although inconclusive, the similarity of shell and matrix δ¹³C values may suggest that Cloudina shells were recrystallized and thickened during diagenesis by a rock-buffered fluid interacting with an organic template. If all Cloudina aggregates are, in fact, proximally sourced detrital assemblages, it is likely that Cloudina lived in ecological niches in or near Ediacaran microbial reefs. Although previous researchers have suggested that Cloudina was not an annelid—largely based on comparisons of shell ultrastructure and the presence of rapidly expanding daughter tubes, neither of which were examined in this study (32)—serpulid communities still may provide an appropriate analog for the sort of ecological niche that Cloudina may have occupied. Today, serpulid reefs consist of tubular, gregarious annelid filter feeders that attach to hard substrates and exist in environments within and proximal to framework reefs. In low- to moderate-energy lagoonal environments, serpulid biocorridences have been shown both to be spatially extensive and to produce fragmented shells remains and sediments (38). An analogous habit for Cloudina—in which Cloudina produced dense aggregations of weakly biomineralized tubes that could be easily broken up—could account for observations of large amounts of detrital skeletal material as well as explain why in situ Cloudina are rare in Ediacaran reef systems.

The observed differences in size (Fig. 4E–H) and morphology (Table 1) between samples must be evaluated with respect to the fact that the aggregates are essentially detritus. It is possible that distinctions between Cloudina populations are entirely due to taphonomy. Preferential size sorting during transportation or burial could explain the variation of diameters between aggregates (35), while diagenetic processes, especially acting on a weakly biomineralized shell or organic structure, could reduce or entirely erase external morphological features such as flared rims or longitudinal crests. However, it is equally likely that the apparent differences between populations are due to taxonomic differences or environmental conditions. Previous researchers divided soft-bodied Ediacara into three spatially and chronologically distinct assemblages representing unique stages of evolution (1). Quantitative 3D observations of tubular Ediacaran biomineralizers may reveal that morphological differences between organisms also represent evolution and that tubes can similarly be subdivided into assemblages by time and space. Unfortunately, both the lack of absolute time constraints and the absence of correlatable features in the δ¹³C chemostratigraphy (Fig. S2C) at Salient Mountain make it impossible to constrain the chronological relationship between Cloudina from Namibia and Canada. Further reconstructions of tubular organisms from throughout the Late Ediacaran to the Early Cambrian, combined with detailed geochronology, may provide an appropriate framework for understanding the evolution of the earliest biomineralizers. Variations in Cloudina size and shape may also reflect the response of the organism to environmental parameters such as nutrient flux, presence of specific microbial textures, and/or different energy regimes. As individual Cloudina aggregates are most likely to be detrital, no one sample or location can provide enough direct evidence to support a link between environment and Cloudina morphology. However, additional studies, consisting of detailed field observations from multiple localities, in conjunction with 3D reconstructions, could provide enough indirect and/or contextual information to determine whether there is a relationship between environmental conditions and the morphology of both Cloudina and other, early, tubular organisms.

Even with extensive field observations and detailed 3D reconstructions, there is a possibility that true in situ Cloudina biocorridences are preserved in the rock record. However, any interpretation of Cloudina as a framework reef builder must contend with the paucity of framework textures in reef outcappings as well as the easily deformed, likely weakly biomineralized, nature of Cloudina tubes. Most importantly, any claim of in situ construction must be evaluated in three dimensions. High-resolution 3D reconstructions enable the extraction of accurate in situ measurements that cannot be collected from 2D cross-sections alone. In this case, 3D in situ measurements allowed for a crucial reinterpretation of the role Cloudina played within Ediacaran reefs. Due to the low density contrast between Cloudina and matrix, this work would not have been possible without the GHI serial sectioning and image-processing pipeline.

**Materials and Methods**

To produce high-resolution orthographic imagery and digital surface models (DSMs) of all three study areas, the Ebee Sensefly, a 0.68-kg, hand-launched, programmable aerial drone equipped with a specially modified 12 megapixel Cannon S110 camera, was used. Multiple flights at each location collected hundreds of overlapping raw red, green, and blue aerial photographs. These images were then geotagged and converted to 12-bit .TIFFs in eMotion, a proprietary flight-planning and processing software package. The processed .TIFFs were loaded into Pix4D, a photogrammetry software package, after which a sparse point cloud was produced by detecting and matching features present in overlapping images. Absolute georeferencing of the dataset was accomplished by matching differently corrected Trimble measurements of control points to features in the sparse point cloud. Following georeferencing, the point cloud was densified and an orthographic image and DSM were calculated. The resulting Driedoornvlakte Farm, Zebra River Farm, and Salient Mountain orthomosaics and DSMs had resolutions of 5.45 cm/pixel, 12.0 cm/pixel, and 21.4 cm/pixel, respectively. A handheld Trimble GeoXH6000 rover without an external antenna was used to collect 1,254 GPS points, arrayed over a survey grid with 10 × 20-m spacing; discrete keywords associated with field observations, including information about texture, lithology, sedimentary structures, and the presence (or absence) of skeletal remains, were recorded in conjunction with each point. The Trimble GPS data were differentially corrected using the GPS Pathfinder Office software package. Data from the TrigNet Springbok base station, 654 km from Driedoornvlakte, were used for differential correction. Horizontal accuracy of the corrected data had a mean of 0.596 m and a SD of 0.126 m, while vertical accuracy had a mean of 0.706 m and a SD of 0.256 m. The corrected data, along with field observations, were then loaded into MATLAB. For each feature of interest (outcrop, microbial facies, skeletal material, and thicket), points were assigned either a 1 if any associated field observations were present or a 0 if no supporting field observations were recorded. Next, experimental variograms were produced using mgstat, a geostatistical toolbox for MATLAB. Then, theoretical variograms were fitted to the experimental data and the best-fit parameters were recorded (Fig. S7). Finally, the entire dataset was processed in mgstat using indicator kriging (39) to produce an interpolated map illustrating the probability of finding the feature of interest within the reef complex. In selected stratigraphic sections, centimeter-scale carbonate samples from approximately every 1.0 m were analyzed for δ¹³C and δ¹⁸O. Each sample was slabbbed, polished, and then selectively drilled. A total of 5 mg of the resulting powder was placed into a borosilicate vial, heated to 110°C to remove any carbonate fraction, then reacted with five drops of HNO₃. The resulting CO₂ was analyzed using a Sercon IRMS coupled with a Gasbench II at Princeton University. The measured precision is ±0.1‰ for carbon and ±0.2‰ for oxygen; all values are reported in delta notation and are relative to the Vienna Pee Dee Belemnite (VPBD) standard.

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Decimeter-scale in situ hand samples of Cloudina and associated organisms were collected for thin sectioning and serial sectioning. Each sample was assigned a unique GPS point using a Garmin GPSmap 64, a Garmin GPSmap 60csx, or a Trimble GeoXH6000 handheld GPS unit and was contextualized with respect to the environment in which it was deposited. Whenever possible, orientation (either bedding plane up or the strike and dip) was marked directly on multiple faces of the extracted sample. Samples designated for serial sectioning and reconstruction were slabbed (~5.4 × 4.0 × 5.0 cm) and mounted using two-part epoxy onto steel plates in preparation for grinding.

The GIRI at Princeton University is a fully automated system that requires minimal operator intervention. GIRI is a computer numerical control (CNC) Mistui MSG-818PC-NC surface grinder which has been retrofitted with a mixing apparatus, retractable rollers, and an imaging stage that consists of two LED lamps and an 80-megapixel Phase One digital back coupled to a 120-mm Schneider Kreuznach macro lens via an electronic shutter. The CNC grinder is capable of moving in 1-µm increments. The camera sensor measures 40.4 × 53.7 mm and contains pixels that are each 5.2 × 5.2 µm in size. When the camera is set up for 1:1 macroimaging, the system is capable of resolving objects as small as 10.4 µm over a 40.4 × 53.7-mm field of view. As the CNC grinder has a high degree of repeatability, samples can be mosaicked to achieve a higher field of view and/or higher resolutions.

The CNC grinder processes a list of machine instructions written in G code, a numerical control language (Fig. 1A). An optimized grinding and imaging routine has been written for GIRI and parameters related to individual samples (i.e., sample name, sample width, step-down size, and number of slices) can be edited and the result saved as a valid G-code file for any given sample. Using a collection of interactive MATLAB scripts and Apple-script functions, the control computer is able to send and receive signals from the grinder, trigger the shutter, and verify image capture, all while GIRI is in operation.

A typical grind cycle begins with the removal of some surface material (as specified by the step size) by a 6-inch diameter diamond or silicon carbide wheel. Following the grind cycle, the sample passes under a roller to clear off any excess fluid and ensure an even sheen. The sample is then positioned under the imaging stage for photographing. At this point, the grinder sends a signal to the control computer requesting an image capture; an Apple-script command triggers the shutter and the image is downloaded into Capture One, a software package designed to work with the Phase One digital back. After the image has been downloaded and verified, the control computer initiates the next grind cycle by sending a signal to the grinder. The process is repeated until the sample has been fully ground and imaged. A typical 1,500-slice sample (at 30 µm per slice) takes 1 wk to grind and image; during this time, the operator needs to replace machine fluids and clean the wipers only every 24 h.

Capture One downloads and stores images as .IIQs, a proprietary raw image file format. While the .IIQ format preserves unedited sensor data—thereby enabling postcapture adjustments of settings such as white balance, exposure, and sharpening—it cannot be opened or processed in programs other than Capture One. Therefore, once a sample has been ground and imaged, the .IIQ file for each slice is adjusted and exported as a 16-bit .TIFF for further processing. The conversion from .IIQ to .TIFF has the downside of dramatically increasing file size by an order of magnitude: A single .IIQ ranges from 50 MB to 80 MB in size while its corresponding .TIFF is approximately 600 MB. As a result, .TIFFs for a typical 1,500-count image stack can consume up to 900 GB of disk space. Due to storage space limitations, and because .IIQ files retain adjustment information, only the .IIQ files are archived after a project is completed.

A user-supervised MATLAB routine was developed to efficiently extract features of interest from .TIFF image stacks. First, superpixels (i.e., regions that consist of pixels that have been clustered together on the basis of color and position, ref. 41) are calculated for each image using the MATLAB superpixels routine. This effectively preserves features edges while oversegmenting the image (Fig. 3A). For each superpixel, a column vector, consisting of pixel color and texture value statistics, as well as the corresponding statistics of superpixels located directly above and directly below the one of interest, is produced and stored. Following this preprocessing, a user defines the classes of interest (e.g., fossil and matrix) and selects representative superpixels for each class within a subset of images from the stack. The resulting collection of classified superpixels is divided into training, verification, and testing datasets, which are then used to train and verify a two-layer feed-forward neural network. The trained network outputs the probability that a superpixel belongs to a given class (Fig. 3B) and predicts the correct class with greater than 90% accuracy. Finally, superpixels are thresholded to produce a binary image for use in visualization and measurement (Fig. 3C).

The stack of processed binary images is loaded into Avizo, a scientific visualization software package. Each slice is given a thickness that corresponds to the amount of material removed during a grind cycle (i.e., the step size), thereby turning each pixel into a volumetric pixel, or voxel.

To isolate and measure individual specimens, a custom graphical user interface (GUI), written in MATLAB, is used. First, a down-sampled stack of the original true color images is loaded into the GUI. Next, a user scrolls through the stack, selects an object of interest, draws a bounding box around the feature, and inputs the first and last slice in which the feature can be found. Finally, in the case of cylindrical objects such as Cloudina, the user marks the center of the object with multiple slices.

The bounding box coordinates, along with information about the first and last slice, are used to extract a subvolume of the larger dataset in Avizo. If center points are provided, a 3D spline is fitted to the points. The length of the spline is treated as the length of the object. Coordinates and tangent values are evaluated at fixed intervals along the spline (for this study, the intervals were at 20%, 40%, 60%, and 80% of the total length of the spline) and the information is used both to produce oriented sections (with the tangent serving as the normal of the plane that defines the sections) in Avizo and to calculate the plunge and trend of the tube.

Semimajor and semiminor axes measurements are made directly on these 2D sections using the built-in ruler tool in Avizo. The cross-sectional eccentricity is calculated by dividing the semiminor axis size value by the semimajor axis size. The rate of semimajor axis size change is defined as

\[(d_i)_{n+1} - (d_i)_n / (d_i)_n\]

where \(d\) is the semimajor axis (in millimeters) and \(n\) refers to the sectional slice number. All means are reported with 1 SD.

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