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Review

Biogenicity and Syngeneity of Organic Matter in Ancient Sedimentary Rocks: Recent Advances in the Search for Evidence of Past Life

Dorothy Z. Oehler [1,*] and Sherry L. Cady [2]

[1] LZ Technology—Jacobs JETS Contract, NASA Johnson Space Center, Mail Code KA, Houston, TX 77058, USA

[2] Pacific Northwest National Laboratory, P.O. Box 999, Richland, WA 99352, USA; E-Mail: sherry.cady@pnnl.gov

***** Author to whom correspondence should be addressed; E-Mail: dorothy.z.oehler@nasa.gov; Tel.: +1-281-483-4259.

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Abstract: The past decade has seen an explosion of new technologies for assessment of *biogenicity* and *syngeneity* of carbonaceous material within sedimentary rocks. Advances have been made in techniques for analysis of *in situ* organic matter as well as for extracted bulk samples of soluble and insoluble (kerogen) organic fractions. The *in situ* techniques allow analysis of micrometer-to-sub-micrometer-scale organic residues within their host rocks and include Raman and fluorescence spectroscopy/imagery, confocal laser scanning microscopy, and forms of secondary ion/laser-based mass spectrometry, analytical transmission electron microscopy, and X-ray absorption microscopy/spectroscopy. Analyses can be made for chemical, molecular, and isotopic composition coupled with assessment of spatial relationships to surrounding minerals, veins, and fractures. The bulk analyses include improved methods for minimizing contamination and recognizing syngenetic constituents of soluble organic fractions as well as enhanced spectroscopic and pyrolytic techniques for unlocking syngenetic molecular signatures in kerogen. Together, these technologies provide vital tools for the study of some of the oldest and problematic carbonaceous residues and for advancing our understanding of the earliest stages of biological evolution on Earth and the search for evidence of life beyond Earth. We discuss each of these new technologies, emphasizing their advantages and disadvantages, applications, and likely future directions.

Keywords: biogenicity; syngeneity; syngenicity; biosignature; biomarker; NanoSIMS; SIMS; Raman; Archean

1. Introduction

Biogenicity and *syngeneity* are the criteria for which every potential organic remnant must be evaluated, regardless of whether the carbonaceous material is preserved in terrestrial or extraterrestrial samples. *Biogenicity* refers to the origin of an organic remnant in a rock from a life form. An organic remnant is potentially biogenic if it resembles other sedimentary organic remains that are well accepted to be of biological origin in morphology and/or geochemistry and if no non-biological processes are known that could have produced that remnant. *Syngeneity* refers to the age of an organic residue compared to the age of the rock in which it occurs. An organic residue is said to be syngenetic, if it is assessed to be the same age as its host rock. Since post-depositional fluid migration can introduce relatively young organic compounds and microbes into ancient rocks, evaluation of the syngeneity is as important as evaluation of biogenicity. Furthermore, it should be noted that the criterion of syngeneity applies also to non-biogenic organic material. It is an independent criterion that is important in the understanding of the origin of any organic material found with ancient rocks.

Paleobiologists and geochemists have long recognized these issues, and there is a significant body of literature aimed at defining criteria that can be used to identify *bona fide* morphological or chemical fossils [1–16]. However, opportunities to evaluate the biogenicity and syngeneity of carbonaceous materials have advanced significantly in the past decade as a result of the application of new analytical and imaging technologies. Recent improvements in detection limits, spatial resolution, and combinations of different chemical imaging and analytical tools are enabling more sophisticated evaluation of candidate biosignatures. These advances have extended the search strategy for evidence of life to include more problematic and fragmentary organic remnants in terrestrial rocks and any that may occur in extraterrestrial materials such as meteorites and rover-based samples.

Paleoarchean sedimentary rocks (>~3.2 billion years in age) illustrate these challenges. Organic material in Archean rocks is commonly more altered than its younger counterparts, e.g., [16,17], as multiple post-depositional processes will have degraded and ultimately destroyed most organic remains over the course of billions of years of geological processing. Consequently, the morphology of preserved organic structures may lack detail (such as cellular features or colonial organization) that could independently support a biogenic interpretation. Chemical composition could aid in assessment of biogenicity, but, until recently, appropriate tools were not available that would allow *in situ* analyses of molecular chemistry that could be correlated with morphology at sub-micrometer-scale scale spatial resolution. In addition, while there is always the possibility that several generations of organic matter can occur in samples of any age, this is a particular hazard in Archean rocks which, because of their relatively altered states, can be crossed by fractures, within which epigenetic, contaminating organic matter could accumulate post depositionally [18,19]. Finally, there is a possibility that organic matter from ancient biological sources could co-exist with organic matter or reduced carbon produced by non-biologic processes, such as that resulting from decomposition of siderite and mantle carbonic

fluids [20], Fischer-Tropsch-Type reactions [21,22], or delivery of abiotic materials to the early Earth by carbonaceous meteorites [23].

To address these challenges, many new analytical techniques have been developed for assessing organic matter in sedimentary rocks, including *in situ* approaches for evaluating micrometer-to-sub-micrometer-scale, organic fragments within their host rocks and new methods for analyzing bulk isolates of organic materials separated from their hosts. The *in situ* approach eliminates the need for harsh chemical treatments typically used to isolate bulk organic fractions and it can be used to interpret individual organic fragments within the context of their spatial relationships to minerals, fractures and veins, and other organic materials in the rock.

Examples of these *in situ* technologies include:

- Confocal Laser Scanning Microscopy—for high resolution, three-dimensional (3D) imaging.
- Raman and Fluorescence Spectroscopy and Imagery—for two-dimensional (2D) and 3D chemical/structural information and thermal maturity.
- Secondary Ion Mass Spectrometry (SIMS)—for carbon and sulfur isotopic composition at micrometer-scale resolution.
- Nano-scale Secondary Ion Mass Spectrometry (NanoSIMS)—for elemental composition (and possible isotopic composition) at sub-micrometer-scale resolution.
- Analytical Transmission Electron Microscopy, utilizing Energy Dispersive X-Ray Spectroscopy (EDS) and Electron Energy-loss Spectroscopy (EELS)—for chemical composition, carbon bonding and crystallinity using electrons.
- Synchrotron-based Scanning-Transmission X-ray Microscopy and X-ray Absorption Near-Edge Structure Spectroscopy (XANES)—for macromolecular structure, crystallinity, chemical composition and bonding, and functional group identification using X-rays.
- Combined Sequential, Focused Ion Beam (FIB)-Scanning Electron Microscopy—for high resolution, 3D imagining of surfaces of carbonaceous residues.
- Two-Step Laser Mass Spectrometry (L^2MS)—for organic molecular structure.

Raman, confocal laser scanning microscopy, SIMS, NanoSIMS, and L^2MS can be used to analyze organic material in petrographic thin sections of rock samples. These sections are typically 30–150 micrometers (μm) thick. In contrast, transmission electron microscopy, scanning-transmission electron microscopy, and synchrotron-based scanning-transmission X-ray microscopy are used on portions of thin sections that require further thinning to thicknesses ranging from 50–250 nanometers (nm). This additional thinning is accomplished either by microtoming with a diamond knife or ion milling with an ion polishing system or a FIB.

The *in situ* techniques can be divided into those that provide 2D information from organic structures exposed at the surface and those that provide 3D information of organic structures by gathering data from various focal points or planes within a thin section. Compared to 2D techniques, 3D techniques are capable of imaging a greater volume of an organic remnant, which can provide potentially useful information at depth within a thin section (such as evidence of branching or folding of individual structures) and information about microbial community constituents (such as their distribution and orientation).

- 2D *in situ* techniques include transmission electron microscopy, scanning-transmission electron microscopy, EELS, synchrotron-based scanning-transmission X-ray microscopy, XANES, SIMS, NanoSIMS, and L^2MS, as well as Raman and fluorescence spectroscopy (which additionally can be used for 3D analysis).
- 3D *in situ* techniques include confocal laser scanning microscopy, Raman and fluorescence spectroscopy, and sequential FIB-scanning electron microscopy. Optical microscopy, while not a new technique, is a critical part of the arsenal of 3D tools, as it provides the basis for almost all of the detailed *in situ* techniques that have been developed in the past decade.

However, there are also disadvantages to *in situ* analyses. For example, as spatial resolution is increased, probe diameters are decreased, and smaller quantities of material are analyzed. Those quantities may be so small that organic molecules may not be detectable or even present within the material analyzed. In addition, SIMS, NanoSIMS, and L^2MS require the volume of interest to be located near the surface of a thin section, which may be a difficult constraint for rare specimens. Finally, *in situ* techniques are time intensive in terms of sample preparation and the need for precise sample mapping to ensure that objects identified in petrographic thin section can be relocated in different instruments when performing the advanced *in situ* analyses.

Thus, the more traditional analyses of the isolates of solvent-extractable and solvent-insoluble (kerogen) organic fractions continue to play important roles in assessment of biogenicity of ancient organic matter. Solvent extracts can contain biomarkers that may reflect the types of organisms present when the sediment was deposited. However, such extracts also can include soluble organic molecules that might have migrated into the sample after it was lithified, and so may contain mixtures of syngenetic and epigenetic materials. Because of this, newer sampling and extraction protocols have been designed to minimize potential contamination [24,25], and innovative approaches have been described that provide methods for recognizing instances when epigenetic organic materials have migrated into sediments after lithification [25].

Kerogen is the organic matter in rocks that is insoluble in acids or other solvents. It comprises the majority of the carbonaceous material in sedimentary rocks (the remainder being the soluble fraction that is extractable with organic solvents). Kerogen is highly complex and does not have a fixed structure or chemical composition. It is comprised mainly of amorphous hydrocarbon macromolecules but can contain trace quantities of elements such as sulfur or nitrogen. The complexity of kerogen has made it difficult to assess, but there have been advances in spectroscopic and pyrolytic techniques that have made it possible to unlock its molecular structure and composition and thereby provide an improved basis for assessment of biogenicity and syngeneity [26,27].

This paper focuses on recently developed techniques and approaches that have been adopted in the paleobiological and astrobiological communities for addressing questions related to the origin of carbonaceous materials in sedimentary rocks. These technologies have yielded new insight into the early evolution of life on Earth, and they offer critical tools that will be needed to advance our understanding of earliest, terrestrial life forms and the search for evidence of ancient life in extraterrestrial materials.

2. New Techniques and Approaches

2.1. In Situ Organic Materials

2.1.1. Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy is used to identify the 3D morphology and potential cellular anatomy of organic structures at sub-micrometer scales [28,29]. When the confocal laser scanning microscope (CLSM) is used to characterize kerogen, fluorescence in the polycyclic aromatic hydrocarbons in kerogen is excited by a laser rastered across the structure at increasing optical depths. The rastering process produces a series of in-focus images that can be computationally integrated to produce 3D renderings of the structure analyzed. While the resolution of this technique is similar to that achievable with traditional optical/fluorescence microscopy (maximum resolution of about 200 nm), the real advantages of confocal laser scanning microscopy lie in the ability to control depth of field, minimize interference from out of the focal plane background that can lead to image degradation, and collect multiple serial sections that can be combined to produce high contrast, in-focus 3D images. Resultant 3D renderings can reveal morphological definition of permineralized microfossils in their host rock to a degree not possible with traditional microscopy [30,31]. Detailed assessments can be made of spatial relationships between the organic structures and enclosing minerals. This technique, when coupled with Raman and fluorescence spectroscopy and imagery (see below), can be used to establish chemical composition and one-to-one correlations between morphology and composition and to distinguish kerogen and abiotically formed, mineral biomorphs, e.g., [32]. These capabilities add to assessments of both biogenicity and syngeneity and have contributed to interpretations of carbonaceous filaments in the ~3.46 billion year old (Ga) Apex chert [29,33].

2.1.2. Raman and Fluorescence Spectroscopy and Imagery

Raman spectroscopy is an analytical technique optimized to detect the changes in wavelength that result from inelastic scattering of laser light by molecules of the material analyzed. Fluorescence spectroscopy is a technique that measures the emission of light from a sample resulting from atomic/molecular absorption of photons in electromagnetic radiation from a laser source and subsequent de-excitation. Both types of spectroscopy can be acquired with the same instrument. Raman and fluorescence have been used for more than two decades to study molecular structure of minerals and carbonaceous matter.

In recent years, Raman spectroscopy has been used to establish the carbonaceous composition of problematic sedimentary material (summarized in [34]) and to investigate changes in carbonaceous matter with burial and metamorphism (which can lead to the use of Raman spectroscopy as a geothermometer [35] and for analyses of thermal maturity and history of alteration [34,36]). The technique allows a one-to-one correlation between chemical information and morphological structures observed in the petrographic thin section. In addition, the technique produces little destruction of kerogen, though, depending on the laser parameters, local heating can be damaging to some types of carbonaceous materials. Raman spectroscopy was used on Precambrian samples to demonstrate a direct correlation between kerogenous composition and permineralized structures in the

770 million year old (Ma) Skillogalee Dolomite, the ~1880 Ma Gunflint Formation, the ~3275 Ma Kromberg Formation, and the ~3465 Ma Apex chert [37]. That work was furthered in 2005 by the combination of Raman spectroscopy and imagery to investigate carbonaceous microfossils in 22 samples ranging in age from ~400 Ma–2100 Ma [34]. A systematic variation in the Raman spectra of (a) the 22 samples (which were also characterized by five independent indicators of geochemical maturity) and (b) a single acritarch, heated progressively from 200–1200 ℃ and analyzed at 16 different points during heating, led Schopf and colleagues to propose the Raman Index of Preservation (RIP) as a new measure of kerogen thermal maturity. The RIP can reveal the presence of epigenetic organic contaminants when they are characterized by significantly different thermal maturity than the bulk kerogen or that inferred from mineral indicators of metamorphic grade. For example, RIP could be used to differentiate between modern or recent endoliths and ancient, syngenetic fossils. In such a case, both the endoliths and the syngenetic fossils would be within the host rock, but the only the syngenetic fossils would be the same age and maturity as the enclosing rock, as could be determined by comparing their RIPs to that of particulate kerogen in the same sample (the modern/recent endoliths would have significantly lower maturity than the kerogen). Conversely, RIP could also support an interpretation of syngeneity when the RIP of a structure is consistent with metamorphic grade of the sample or the RIP of the bulk, particulate kerogen.

Raman, fluorescence and confocal laser scanning microscopy provide complementary types of information and together can be used to assess micrometer-scale structure, chemistry, and molecular composition of organic residues and associated minerals [29–37] and biominerals [38].

2.1.3. Secondary Ion Mass Spectrometry (SIMS)

SIMS is a technique whereby an ion beam is used to sputter ions from the surface of a solid specimen and the ejected secondary ions are collected and analyzed by a mass spectrometer. Data collected provide information on elemental and isotopic composition of the uppermost layers of the specimen. The first work to show that SIMS could be used to determine, *in situ*, the $\delta^{13}C$ of individual microfossils was that by House *et al.* in 2000 [39]. That work concentrated on analysis of microfossils in the Proterozoic Bitter Springs (~830 Ma) and Gunflint (~1880 Ma) formations and demonstrated the veracity of the use of SIMS for *in situ* isotopic measurements by comparing the SIMS-derived $\delta^{13}C$ values (−20 to −30‰ for 14 of 15 measured individual specimens) to the values previously measured on bulk samples of kerogen in the same rocks (−21 to −30‰ for 31 of 32 bulk samples [40]).

Subsequent studies have reported SIMS-based results from Archean deposits. A 2007 SIMS study by Van Zuilen *et al.* [17] of organic matter in 3.2–3.4 Ga Archean rocks from the Barberton Mountain Land looked at carbon isotopic composition and nitrogen/carbon ratios of organic matter in several different formations. That study noted that hydrothermal alteration can cause small scale migration and re-deposition of organic matter, and it concluded that the organic matter in the cherts analyzed is likely to be metamorphosed biological material.

An attempt in 2013 by House and colleagues to acquire SIMS-derived $\delta^{13}C$ values of different Archean structures was also successful [41,42]. This study focused on organic microstructures from the ~3 Ga Farrel Quartzite of Australia. Those structures appeared to be composed of relatively dense

material in optical microscopy, and as demonstrated by Raman spectroscopy [14], they are composed of kerogen and likely to represent *bona fide* microfossils (a conclusion consistent with morphological interpretations [43] and NanoSIMS element distributions (see below) [44]. Results from the 2013 SIMS study were three-fold: First, by showing a $\delta^{13}C$ difference between background organic matter and spindle-shaped structures in the same thin section, the SIMS data supported the concept that the Farrel Quartzite spindles are *bona fide* microfossils—and not pseudofossils formed by the aggregation of organic debris. Second, the weighted mean $\delta^{13}C$ value of −37‰ of the analyzed spheroids and spindles supported earlier conclusions that the structures are biogenic and suggested further that the structures are likely to be remnants of autotrophic organisms. Third, the tight clustering of the isotopic data for the spindle-shaped forms—at values significantly more depleted in ^{13}C than background—supported an interpretation that the spindle-like structures are remains of planktonic microorganisms that lived in open water (a conclusion also reached from their morphology [43,44]) where they could grow without CO_2 limitation and, as a result, develop maximal isotopic fractionation. Thus, the application of SIMS ultimately revealed that the Farrel Quartzite organisms were likely to have been planktonic autotrophs. Other spindle-shaped forms in Precambrian sedimentary rocks were first reported by Walsh in 1992 [45] from the older, ~3.33–3.45 Ga Kromberg Formation of South Africa, and more recently, apparently similar forms have been reported from the ~3.35–3.43 Ga Strelley Pool Formation of Australia [46,47]. Work is in progress to analyze the *in situ* $\delta^{13}C$ values of the spindle-like structures in each of these older deposits and to compare the results to those from the Farrel Quartzite specimens [42].

SIMS was used by Lepot *et al.* [19] in a detailed study designed to derive H/C ratios and $\delta^{13}C$ values of the organic constituents found in rocks from the Strelley Pool Formation. Their SIMS results, when added to Raman spectroscopy and petrographic observations, suggest that the Strelley Pool organic constituents and possible microfossils are biogenic and syngenetic with the Archean cherts in which they are found. Their work further shows that the Strelley Pool Formation includes a wide range of $\delta^{13}C$ values that can be correlated with organic textures. Some of those textures are in late stage vein-filling material; other textures are associated with possible microfossils, and results suggest that diagenetic migration of organic matter in those materials has been on a millimeter scale. Spindle-like, lensoid structures appear to be comprised of two types of organic matter: relatively high H/C and $\delta^{13}C$ material that makes up the organic network of these structures and lower H/C and $\delta^{13}C$ material that makes up associated, carbonaceous globules. Lepot *et al.* [19] noted that these heterogeneities could either reflect different types of carbon-fixation metabolisms or selective preservation.

Williford *et al.* [48] conducted a SIMS-based $\delta^{13}C$ study of a suite of Proterozoic microfossils in stromatolitic cherts from the Gunflint, Bitter Springs, Chichkan (~775 Ma), and Min'yar (~740 Ma) formations. They reported a range of $\delta^{13}C$ results that are consistent with photoautotrophic carbon fixation via ribulose bisphosphate carboxylase, which is typical of cyanobacteria and photosynthetic eukaryotes. Intracellular isotopic heterogeneities were observed, suggesting that carbon isotopic composition of individual microfossils may be influenced by preservation of specific biomolecules. In addition, replicate measurements of a likely colonial cyanobacterium and an adjacent leiosphaerid acritarch showed distinctive $\delta^{13}C$ values that could reflect the difference between cyanobacterial and eukaryotic photosynthesis. In sum, this study showed that *in situ* isotopic analyses of ancient,

permineralized microbial mats can be used to distinguish biological affinities and metabolic fingerprints within complex microbial communities that span a significant range of ages.

In all of these studies of SIMS-based $\delta^{13}C$, there has been recognition that isotopic composition alone cannot imply biogenicity, as recent studies have shown that various abiotic processes can produce isotopically light organic compounds [49,50]. However, these recent studies have combined isotopic data from SIMS with a variety of additional analyses to add to the assessment of biogenicity (e.g., morphology, population distributions, Raman spectroscopy, and element distributions determined with NanoSIMS). These combined studies illustrate the importance of utilizing a variety of analytical tools for evaluation of organic materials that occur in ancient rocks.

Finally, SIMS also has been used to assess sulfur isotopic composition of the sulfur associated with organic structures in stromatolites of the Strelley Pool Formation [51]. Results were interpreted as reflecting sulfurization of organic matter by H_2S in pore waters associated with microbial mats and potentially involving microbial sulfur metabolism.

2.1.4. Nano-Scale Secondary Ion Mass Spectrometry (NanoSIMS)

NanoSIMS is a secondary ion analysis technique, utilizing an instrument that has been optimized for nano-scale spatial resolution. The technique enables determination of elemental composition at resolutions that approach 50 nm, a resolution that is 10–50 times greater than that attainable with conventional SIMS or electron microprobes. NanoSIMS also offers extremely high sensitivity (ppm to ppb range for some elements) at high mass resolution, a range almost an order of magnitude greater than can be achieved with EDS. The NanoSIMS instrument also has a rastering capability that allows acquisition of ion images and this allows a one-to-one correlation of element maps with microstructures observed in optical microscopy.

This technique was first applied to ancient microfossils in a feasibility study by Robert *et al.* in 2005 [52]. In 2006, a follow-up study was carried out by Oehler and the Robert group [53] on well preserved and non-controversial organic microfossils from the ~830 Ma Bitter Springs Formation of Australia. Results demonstrated that sub-micrometer-scale maps of metabolically important elements (carbon (C), nitrogen (measured as CN), and sulfur (S)) could be correlated with kerogenous microfossils identified by optical microscopy. The data also showed that spatial distributions of C, CN, and S in the individual microfossils are nearly identical and that the variations in the concentrations of these elements parallel one another. In element maps, C, CN, and S appear as globules that are aligned to form what Oehler *et al.* [53] interpreted as remains of walls and sheaths of fossiliferous structures. Since nitrogen is a biologically fixed element, the fact that the concentration of nitrogen mirrors that of carbon would be consistent with a biological origin for the nitrogen—an interpretation supported by a lack of such correlation between nitrogen and carbon in organic material that fills secondary veins, as discussed below. The silicon (Si) and oxygen (O) maps obtained in the 2006 study illustrated an intimate association of Si and O with C in the microfossils. The authors subsequently suggested that this relationship reflects the process of silica permineralization of biological remains and provides an indicator of syngeneity of the fossilized material with the mineral matrix [18]. NanoSIMS characterization of these undisputedly biogenic Bitter Springs microfossils serves as a baseline for interpretations of

less well preserved structures and fragments of carbonaceous material that might occur in older or extraterrestrial materials.

An example of such an application is provided by NanoSIMS analyses of controversial carbonaceous structures preserved in the ~3 Ga Farrel Quartzite [18,44]. This deposit includes spheroidal and unusual, relatively large, spindle-shaped structures. The latter, in particular, upon initial petrographic examination, appeared likely to be pseudofossils of organic debris surrounding possibly pre-existing minerals. NanoSIMS data from the Bitter Springs microfossils and from carbonaceous material filling a secondary vein in cherts from the older Strelley Pool Formation provide comparative datasets for analyses of the Farrel Quartzite structures. Results showed clear differences between the organic residues in Farrel Quartzite and that in the secondary vein in the Strelley Pool Formation.

For example, for the Farrel Quartzite spindles, coincident accumulations of C, CN, and S and a direct relationship between the concentrations of C and CN are consistent with a biogenic interpretation. In contrast, the secondary vein material showed accumulations of C and CN that were not coincident and showed an inverse relationship between concentrations. Also, the Si and O distributions in the Farrel Quartzite structures showed the intimate spatial relationship with C and CN that was suggested to be associated with permineralization and syngeneity, as discussed above, but the Si and O distributions in the secondary vein did not show that same intimate spatial relationship to C and CN in the vein. These results support the biogenic and syngenetic interpretation for the spheroids and the spindle-shaped structures in the Farrel Quartzite, and this conclusion has added to arguments that life on the early Earth was relatively diverse [44].

NanoSIMS has been used to assess element concentrations in organic material associated with other types of Archean deposits including stromatolites in the Strelley Pool Formation [54–57] and stromatolitic microbialites in the ~2.73 Ga Tumbiana Formation of Australia [57]. The technique has additionally been used to investigate $\delta^{13}C$ of organic fragments in early Precambrian organic matter [55,58]. However, as the quantities of carbon assessed with NanoSIMS are quite small, the error bars in NanoSIMS-derived $\delta^{13}C$ analyses are relatively large. Consequently, it may be that determination of $\delta^{13}C$ of ancient organics with SIMS may provide more meaningful results, as SIMS has broader areas of investigation and deeper depths of penetration [59] that enable larger volumes of material to be analyzed (with greater quantities of carbon), such that signal-to-noise ratios are improved and error bars are minimized.

2.1.5. Analytical Transmission Electron Microscopy

In 2004, Moreau and Sharp used a transmission electron microscope in the scanning mode (STEM) and energy dispersive X-ray spectroscopy (EDS) to analyze, *in situ*, organically preserved Precambrian microfossils [60]. In that work, well accepted microfossils (*Huroniospora* spp.) from the ~1880 Ma Gunflint Formation were ion thinned to electron transparency (~100 nm) and then studied with STEM and EDS. Results clearly identified the intimate relationship between the kerogen of *Huroniospora* coccoidal microfossils and silica of the enclosing chert. The authors concluded that the organic material of the Gunflint microfossils promoted nucleation of opaline silica and that further diagenesis produced submicroscopic silica spheroids in intimate spatial association with organic remains. They suggested that the silica spheroids may themselves constitute a mineralogical biosignature. Additional microanalysis,

with electron energy-loss spectroscopy (EELS) and electron loss near-edge structure (ELNES), illustrated a marked difference between Gunflint kerogen and graphite samples. The kerogen appeared to be amorphous and did not display any crystallinity, as would be expected in graphite (formed abiotically) or in kerogen altered as a result of metamorphism. Based on these results, Moreau and Sharp inferred that the Gunflint kerogen is derived from degraded cellular material rather than disordered graphite or abiotically formed carbon.

In 2012, Wacey *et al.* [61] applied traditional transmission electron microscopy to *in situ* organic structures in FIB sections from the Strelley Pool Formation and the Gunflint Formation. The transmission electron microscopic images of all samples showed the permineralized structures in both formations to have been greatly altered by nucleation of silica.

2.1.6. Synchrotron-Based Scanning-Transmission X-Ray Microscopy Coupled with X-Ray Absorption Near-Edge Structure Spectroscopy (XANES)

Investigation of carbonaceous material in the ~3.46 Ga Apex chert by De Gregorio and Sharp [62] utilized a combination of transmission electron microscopy, ELNES, scanning-transmission X-ray microscopy, XANES, and SIMS to assess biogenicity of potential microfossils. The authors compared their results with similar analyses of both accepted microfossils of the Gunflint Formation and abiotic carbon compounds formed from Fischer-Tropsch-Type (FTT) reactions. The results of this study indicate that the aromatic content of the carbonaceous material in the Apex chert varies less than it does in Gunflint microfossils and the ELNES spectra of organic residues in the Apex chert are more comparable to amorphous carbon than graphite. It is worth noting that the authors could not exclude the possibility that the Apex material could have been produced by FTT reactions in a hydrothermal vent. Additional efforts to elucidate macromolecular hydrocarbon structure, bonding, functional group chemistry, and potential biotic elements (nitrogen, phosphorous, sulfur) of the organic residues in the Apex chert relied on scanning-transmission X-ray microscopy, XANES, and SIMS [63]. The findings of this latter study support the hypothesis that the organic remains in the Apex chert are similar to Gunflint kerogen. Though an abiotic source could still not be ruled out in this second molecular-level study, the authors argued that it would be unlikely that abiotic syntheses could recreate both the structural and compositional complexity (including the biotic element abundance) of the Apex carbonaceous matter since such a process is not known. This same approach, when used to assess molecular-level characteristics of carbonaceous material preserved in the Strelley Pool Formation of Australia (~3.35–3.43 Ga), led the authors to a similar conclusion: the residual organic material has molecular characteristics more consistent with a biotic than an abiotic origin [64].

In these latter studies [63,64], chert samples were crushed to a powder and small grains of chert in that powder were selected for study, then embedded in molten sulfur and thinned by microtoming with a diamond knife to thicknesses of 90–200 nm. This technique [65] minimizes potential alteration or contamination of carbonaceous material that might occur if prepared via ion beam sectioning, and it enables investigation of carbonaceous material *in situ* within the chert matrix. However, using this technique, it was not possible for the authors to link their results directly to the putative microfossils observed in petrographic thin sections via optical microscopy [33,66]. In other studies, scanning-transmission X-ray microscopy, combined with XANES has been used to characterize the

molecular nature of specific fossils and types of kerogen identified by optical microscopy and thinned by ion-beam milling with a FIB for true *in situ* analysis [67–69]. Results revealed functional groups that were thought to be related to biological composition and diagenesis. In Lepot *et al.*, 2009 [70], results from Raman, scanning-transmission X-ray microscopy, and scanning and transmission electron microscopy show a correlation of calcified layers and organic sulfur suggestive of a role of bacterial sulfate-reduction in the precipitation of calcium carbonate, a view consistent with the concept that precipitation of carbonate in stromatolites may be influenced by bacterial processes.

A combination of several of the above techniques (optical microscopy, confocal laser Raman spectroscopy, scanning electron and high resolution transmission electron microscopy of microtomed samples, scanning-transmission X-ray microscopy, XANES, and transmission electron microscopy of FIB sections) has been used to evaluate potential biogenicity of graphitic particles in ~3.80 Ga rocks from West Greenland [71]. The results support previous conclusions that graphitic particles are associated with apatite grains [72] and that more than one generation of graphitization could have occurred. Similarly, recent work illustrates that multiple generations of organic material may be present in the Apex chert [73]. These sorts of complexities underscore the need for *in situ* study of sedimentary organic matter along with detailed assessment of spatial relationships of the organic matter to surrounding minerals, veins, and fractures.

2.1.7. Combined Sequential, Focused Ion Beam (FIB)-Scanning Electron Microscopy

Scanning electron microscopy of sequential FIB sections to produce 3D images of surfaces was introduced by Schiffbauer and Xiao in 2009 [74] in their study of ~1 Ga microfossils from China. The 2012 study by Wacey *et al.* [61] continued this type of work on carbonaceous residues in both the Gunflint and Strelley Pool Formations. They took sequential FIB sections, each separated by 75–200 nm, and imaged the sections with scanning electron microscopy to produce tomographic renderings at an average of 30 nm/pixel in the x and y directions [75] which would yield a resolution of about 90 nm in the x and y directions. Resolution in the z direction varies with slice spacing. The renderings showed the surfaces of the structures with depth in the section. For the Gunflint microfossil, they obtained 220 FIB slices, each ~15 μm × 15 μm. Compared to confocal laser scanning microscopy, the resolution using this technique is higher, but the technique is limited to surface images and would not illustrate features of cellular anatomy that might be preserved within microfossils. The FIB technique is also destructive to the sample, and the surface images produced would have gaps for the separation between each FIB section. Nevertheless, this approach can yield a new body of morphological information at very high resolution that may be applicable to assessment of the origin and history of ancient carbonaceous materials.

2.1.8. Two-Step Laser Mass Spectrometry (L^2MS)

The L^2MS technique, which provides *in situ* analysis of molecular constituents of organic matter, has been used since the 1990s to assess aromatic compounds in meteorites. The technique uses a laser to access surface-bound organic species. Two laser pulses are used, the second orthogonal to, and separated in time from, the first. Both neutral (desorbed) species and ionized products are produced and then extracted and analyzed in a time-of-flight mass spectrometer which separates the

desorbed and ionized products temporally and spatially. Recent advances in ionization mechanisms allow for molecular identification and spatial mapping of a greater variety of organics species [76–79]. Though L^2MS was used in 2001 to study polycyclic aromatic hydrocarbons in ancient terrestrial rocks [79], the technique has been used primarily for analyzing extraterrestrial materials (e.g., meteorites, interplanetary dust, and cometary dust particles). Recent enhancements in the technology, however, may expand the applicability of L^2MS to more investigations of organic compounds in early Earth materials. Challenges in using this technique are associated with potential organic contaminants in terrestrial or extraterrestrial materials and the possibility that some of the laser-desorbed or ionized products might be produced from such contaminants.

2.2. Isolates of Kerogen and Soluble Organic Materials

2.2.1. Kerogen

Kerogen comprises the majority of the organic matter in sedimentary rocks. In the early years of Precambrian paleobiological studies, particulate kerogen was considered to be syngenetic with its host rock, particularly when found in cherts, where the interlocking microcrystalline quartz structure was thought to isolate syngenetic organics from post-depositional contaminants that could have entered the rock from migrating fluids. Subsequent work, however, showed that migrating organic compounds can be absorbed "irreversibly" onto kerogen such that the adsorbed compounds cannot be removed by exhaustive solvent- or water-based extractions [80]. Such materials conceivably could come into contact with kerogen, even in a chert, via fractures or at grain boundaries. Thus, while kerogen is initially syngenetic with deposition of its host rock, that kerogen can be altered subsequently by post depositional, migrating fluids and may contain both syngenetic and epigenetic constituents. Follow-up work, attempting to remove potentially adsorbed contaminants from kerogen, has demonstrated that a relatively low temperature step of hydro-pyrolysis (pyrolysis under hydrogen gas pressure) can remove adsorbed hydrocarbons from kerogen [81]. If that first step of pyrolysis is then followed by a higher temperature step, compounds attached to kerogen by covalent bonds are released. This covalently bound material is considered to be the syngenetic material in kerogen [81].

Syngenetic components of kerogen were investigated in the study by Derenne *et al.* [26] that used solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy and Curie point pyrolysis-gas chromatography/mass spectrometry to evaluate kerogen from the ~3.46–3.47 Ga Warrawoona chert. The authors found that the Warrawoona organic matter (a) lacks evidence of severe, thermally-induced chemical modification (from the NMR spectra), and (b) contains a homologous series of long-chain aliphatic hydrocarbons consistent with a biological origin (*i.e.*, the slight odd-even carbon number predominance in the pyrolysate is not characteristic of abiotically produced aliphatic hydrocarbons). The study further revealed that the aliphatic chains they uncovered are covalently bonded to the kerogen. The authors concluded that the aliphatic compounds that they detected by pyrolysis are syngenetic with the Warrawoona chert. Similar work was carried out by Marshall *et al.* [27] on kerogen in ~3.35–3.43 Ga cherts from the Strelley Pool Formation. Again, covalently bonded material in hydro-pyrolysates was considered to be syngenetic with the cherts, and conclusions support a biogenic origin for that material. However, this study found that the kerogen in the Strelley Pool

Formation was virtually all aromatic. Discrepancies between the mainly aromatic kerogen in the Strelley Pool Formation [27] and the aliphatic constituents found in the Warrawoona kerogen [26] should be evaluated further to assess whether they may be related to the specific techniques used or whether they reflect true differences attributable to unique depositional or post-depositional histories of the Warrawoona and Strelley Pool samples.

2.2.2. Soluble Organic Materials

Detailed analyses of soluble organics from Neoarchean sedimentary rocks were reported in a study by Waldbauer *et al.* [25] in 2009. That work emphasized the importance of determining syngeneity, and five lines of evidence were used to address this question. The investigation centered on analyses of two cores from the Agouron Griqualand Drilling Project in southern Africa. Those cores recovered more than 2500 m of sedimentary rocks from the Transvaal Supergroup, with depositional ages between 2.46 and 2.67 Ga. The Transvaal Supergroup is made up of a siliciclastic-carbonate ramp and includes peritidal, as well as platform, slope, and basinal facies. The two cores were drilled 24 km apart and were correlated to each other by volcanic and impact spherule layers, distinctive facies, and bulk geochemistry of the shales.

Extracts of soluble organic materials were acquired from equivalent intervals in each of the cores with the use of clean drilling, handling, sampling, storage and analyses protocols, details of which are described in [25]. Blanks were used to assess the possibility of procedurally introduced or laboratory contaminants.

The five lines of evidence used to assess potential post-depositional contamination in the cores included:

1. Thermal maturity: To be syngenetic, all constituents of the extracts must have compositions indicative of a level of maturity consistent with that of the host rock. In the Transvaal study, the extracts have compositions consistent with a wet-gas zone of thermal cracking, a conclusion consistent with the prehnite-pumpellyite to lower greenschist metamorphic grade of the host rocks.
2. Absence of petrochemical signatures suggestive of man-made contaminants or Phanerozoic molecular signatures.
3. Similarity of extracts from the two cores in bed-to-bed comparisons, which suggests depositional control on extract composition, rather than control by man-made contamination or that produced from migrating fluids in the subsurface.
4. Similarity of extracts obtained before and after the samples were treated with hydrochloric and hydrofluoric acids to dissolve mineral matter. Since extracts obtained after acid treatment represent materials likely to have been trapped within minerals, these extracts are less likely to have been exposed to migrating fluids or laboratory/drilling contaminants than the extracts obtained before acid treatment. So, where extracts before and after acid treatment are similar, they are most probably derived from syndepositional organic matter.
5. Dissimilarity in composition and maturity between extracts from the Archean samples and the overlying Dwyka Formation (a Permo-Carboniferous diamictite). This dissimilarity provides evidence that the extracted organic compounds in the Archean section of the cores are depositional signals of Archean age and not younger contaminants.

This study provides a new basis for assessing syngeneity and suggests that syngenetic molecular fossils can be preserved in ancient detrital sediments. Nevertheless, some doubt may be cast on this type of biomarker work, as a newer study of cores drilled in 2012 in the Australian Pilbara, using even more stringent protocols, failed to find detectable alkanes, hopanes, steranes, or triterpanes [82]. Whether this result extends to Archean biomarker work from other localities is not clear but will certainly be an area of continued research.

3. Discussion

This paper focuses on sedimentary organic residues and the technologies that have gained prevalence in the past decade for assessing their origin and significance. Advances in both *in situ* techniques and analyses of bulk isolates of kerogen and soluble extracts are described and the ways in which these new technologies have been applied are demonstrated. We have not addressed potential mineral biosignatures or organo-sedimentary structures (such as stromatolites), as those fields of inquiry involve different sets of questions and analytical techniques. However, to the extent that many (bio)minerals and stromatolites preserve organic residues that can be evaluated independently, all of the techniques discussed herein apply.

Traditionally, efforts to assess biogenicity and syngeneity of organic residues in sedimentary rocks have focused on geochemical signatures in kerogen and soluble extracts and/or morphological and chemical signatures of organic materials observable in thin section using optical microscopy and techniques such as Raman spectroscopy. Objectives were to define features likely to be biogenic (e.g., specialized cell types known in modern biology, evidence of cell division in paired unicells [83,84], colonial organization, chemical and isotopic signatures suggestive of living systems, and spatial distributions of carbonaceous remains suggestive of biologic origin such as association with potential biofilms and mat-type architectures [9,10,13]). However, the earlier geochemical investigations lacked the refinements discussed in Waldbauer *et al.* [25] and French *et al.* [82] for addressing syngeneity of soluble extracts as well as those introduced by Derenne *et al.* [26] and Marshall *et al.* [27] for elucidating molecular composition of syngenetic components of kerogen. Similarly, the previous body of studies lacked application of the newer technologies for *in situ* analysis of micrometer-to-sub-micrometer-scale chemical structure and elemental/isotopic composition.

Cady *et al.* in 2003 [10] discussed the use of high resolution transmission electron microscopy, Raman spectroscopy, ion microprobe analyses, synchrotron X-ray tomography, and time of flight-SIMS, and they stressed the value of optimizing these techniques to acquire multiple characteristics of the same structure. To a large extent, the last decade has seen that sort of advancement. Researchers have realized the limitations of trying to determine biogenicity solely on a single type of analysis. Accordingly, recent strategies for demonstrating biogenicity have linked chemical, isotopic, and molecular signals with morphological evidence for life. These correlative approaches have improved our understanding of the nature and potential origin of problematic and controversial structures and material. For example, in 2007, McKeegan *et al.* [72,73] used Raman imagery to create a high resolution, 3D map of a quartz-enclosed, apatite-hosted graphite particle in >3830 Ma Akilia supracrustal rocks from West Greenland. They used that map to characterize the spatial relationships of that inclusion to the surrounding minerals prior to SIMS analysis for $\delta^{13}C$. This combination of data

allowed confirmation of the existence of isotopically light graphitic particles in the Akilia sample. Another example is the 2013 work by Lepot *et al.* [19] who used combinations of Raman spectroscopy, petrography, and SIMS for both H/C and $\delta^{13}C$ to conclude that organic constituents in the ~3.35–3.43 Ga Strelley Pool Formation are most likely biogenic and syngenetic. Finally, Cady *et al.* [10] also singled out biotic trace element distributions as a new criterion for establishing biogenicity, and it was the identification of biotic trace elements coupled with macromolecular organic structure that led De Gregorio and colleagues to favor a biogenic origin for organic structures in the ~3.46 Ga Apex chert [63,64].

The advances in *in situ* analyses should be especially useful in assessing poorly preserved, fragmentary organic residues that might be found in some of Earth's oldest, Archean rocks and potentially in extraterrestrial samples. Such materials are unlikely to exhibit the excellent organic preservation known from many Proterozoic cherts in which delicate cellular structures are three-dimensionally permineralized by the silica. These newer techniques enhance our abilities to identify the chemistry of minute carbonaceous fragments and, at the same time, determine the spatial relationships of such fragments to surrounding mineral and organic constituents—both aspects being key components of a strategy to assess the biogenicity and syngeneity of organic matter in geological materials.

4. Future Directions

Many of the techniques discussed by Cady *et al.* in 2003 [10] have matured over the past decade. Others, such as NanoSIMS, SIMS-based isotopic composition of individual microstructures, confocal laser scanning microscopy, and 3D Raman and fluorescence spectroscopy are relatively new in paleobiological or astrobiological studies and are being used in new applications. While transmission electron microscopy of FIB sections is also relatively new, the study by Wacey *et al.* [61] illustrates that the ultrastructure of organic structures is greatly affected by silicification. This effect was also seen in ultrastructural studies of excellently-preserved microfossils from the Neoproterozoic Bitter Springs Formation [85]. These observations may suggest a limited utility for ultrastructure in some ancient, permineralized microfossils. The FIB-scanning electron microscopy study of Wacey *et al.* [61] illustrates 3D images of surface structures, reminiscent of images obtained with confocal laser scanning microscopy, and the combination of these techniques with *in situ* chemical and molecular analyses are likely to provide new avenues for future study. Scanning-transmission X-ray microscopy and kerogen hydro-pyrolysis/spectroscopy are among the newest techniques, and procedures are still being assessed and refined. L^2MS has been used mainly in meteorite studies, but with its recently increased sensitivity and resolution, this technique may find future applications in studies focused on *in situ* molecular signatures of ancient terrestrial organic materials.

Perhaps, one of the most interesting and challenging results in recent years is the conflicting evidence for syngeneity of Archean biomarkers. The 2014 report by French *et al.* [82] on core samples from the Pilbara failed to detect alkanes, hopanes, or steranes. Those cores and samples were acquired using the most stringent protocols. Moreover, analysis of a core drilled in earlier years from the same formation demonstrated an abundance of hydrocarbons, now attributed to likely contamination introduced during drilling, sampling, and storage. These results cast doubt on other studies of ancient biomarkers and bring into focus the controversy that has existed for some years regarding the

syngeneity of Archean molecular fossils in soluble extracts (e.g., compare [86] with [87,88]). Nevertheless, it is conceivable that other localities may have been richer in organic matter originally, and/or that they experienced geologic histories that were more favorable to preservation of syngenetic organic materials. Clearly, this will be an area of future investigation that should lead to new protocols for sample collection and analysis as well as insight into the importance of optimal depositional settings and geologic histories for the preservation of syngenetic biomarkers. The recent work [26,27] on kerogen pyrolysis does imply that syngenetic biomarkers exist and can be detected in some types of Archean rocks. While there is some disagreement over interpretations (e.g., [27]), we expect this approach to be applied to many more deposits in the near future. In fact, assessment of syngenetic biomarkers in kerogen may play a significant role in distinguishing biomarkers in soluble organic fractions and separating syngenetic constituents from post-depositional contaminants.

Future investigations should also include the search for potential organic biosignatures in sedimentary rocks other than chert. Cherts are chemical precipitates in which organic microfossils with high morphological fidelity can be preserved by permineralization. Accordingly, most of the information we have about Precambrian life comes from ancient chert deposits. However, cherts represent only one type of sedimentary facies. Other rock types represent different environments of deposition and may include remnants of biological communities that are not preserved in cherts. For example, shales are clastic sedimentary rocks that are more common than chert in the geologic record on Earth and they can be rich in organic matter. While they may be more susceptible to post-depositional contamination by migrating fluids (since they lack the interlocking grain structure of chert that can isolate syngenetic organic materials), their high organic carbon content makes them reasonable candidates for applying the advanced geochemical evaluation methods to assess the biogenicity of kerogen and soluble extracts. Moreover, even though most shales lack structurally preserved, organic microfossils recognizable by optical microscopy, some Archean grey shales and siltstones preserve surprisingly large spheroidal, organic microfossils [89] that illustrate a previously unknown microbiota, presumably unique to the grey shale/siltstone facies. Accordingly, even though abrasion, compression, and bacterial degradation during clastic deposition and burial may physically damage many morphological remains of ancient organisms in many shales, it is likely that small fragments of syngenetic microorganisms may exist within a variety of Archean siliciclastic deposits. The newer *in situ* techniques would be ideal for analyzing such fragments of organic matter. Additionally, because shales and siltstones represent sedimentary facies that are distinct from that of cherts, studies of organic residues in these types of sedimentary rocks should provide insight about the biological constituents of different ecological niches, adding to our understanding of the diversity of life on the early Earth. Similarly, other rock types, such as gypsum [90], impact glasses [91,92], vesicular pillow basalts [93], and even some metamorphic rocks [94] which can preserve organic fossils on Earth, should be evaluated as targets in the search for evidence of earliest terrestrial life or potential extraterrestrial life.

The techniques described above could play important roles in assessing the origin of organic matter that may exist in meteorites. Though organic matter in carbonaceous meteorites is abiotic, that does not preclude the potential for biogenic organic matter to be preserved in stony meteorites from other planetary bodies, such as Mars. While the origin of such organic matter will be controversial, the key to its evaluation will lie in studies using a combination of *in situ*, micrometer-to-sub-micrometer-scale techniques for assessment of elemental composition, chemical structure, molecular constituents,

morphology, and carbon and hydrogen isotopic composition e.g., [50,95]. In addition, establishing syngeneity of such organic carbon in meteorites will be equally critical because of the possibility that meteorites can be contaminated with terrestrial microbes or soluble organics. Meteorites, in their fall to Earth, may preferentially break along pre-existing fractures or planes of weakness that could have been sites of migrating fluids in an extraterrestrial setting. Such features would be interesting because they could contain remains of potential extraterrestrial, endolithic life forms. Caution is warranted, however, as fractures and weak zones will also be preferential sites for Earth-based fluid incursion and invasion by terrestrial microbes. Discrimination between terrestrial contaminants and organic matter of potential extraterrestrial origin will be facilitated by many of the advanced techniques discussed in this review.

5. Conclusions

The toolkit for assessing biogenicity and syngeneity of organic residues in sedimentary rocks has grown considerably in the past decade. A variety of new technologies now exists for both *in situ* organic analysis and assessment of bulk isolates of kerogen and soluble organics. The *in situ* techniques can play a critical role in establishing composition and spatial context of micrometer-to-sub-micrometer-scale organic residues while the advanced methods for analysis of kerogen and soluble organics have the potential to reveal previously undeterminable syngenetic biomarkers that can provide new insights into metabolism and evolutionary relationships of early life on Earth. Future refinement is expected particularly in the areas of SIMS for isotopic studies, L^2MS, kerogen pyrolysis, and kerogen analysis by synchrotron-based scanning-transmission X-ray microscopy. Organic matter isolated from rock types other than chert (e.g., shales and siltstones, gypsum, volcanics, and impact glasses) is likely to play an increasingly important role in our understanding of early terrestrial life. Continued study of biomarkers in Archean shales should clarify the prevalence of contamination in ancient shales, and guidelines are likely to be defined that will aid in sample selection and methods of analysis that will maximize recovery of syngenetic, soluble organic components. Studies using combinations of analytical methods will be particularly important in determining the significance of problematic and controversial organic materials. These are the sorts of materials that commonly occur in some of Earth's oldest rocks and that might be found in extraterrestrial materials in the course of searching for evidence of life beyond Earth. We expect that the next decade will bring a host of new discoveries based on many of the techniques described herein, and that these discoveries will provide new insight into the origin and early evolutionary history of life on Earth and the potential existence of life beyond Earth.

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Author Contributions

Dorothy Z. Oehler conceived of this paper and provided details of recent studies. Sherry L. Cady contributed historical perspective and added details for techniques. Both authors contributed to the writing.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Schopf, J.M. Modes of fossil preservation. *Rev. Palaeobot. Palynol.* **1975**, *20*, 27–53.
2. Schopf, J.W.; Walter, M.R. Archean microfossils: New evidence of ancient microbes. In *Earth's Earliest Biosphere: Its Origin and Evolution*; Schopf, J.W., Ed.; Princeton University Press: Princeton, NJ, USA, 1983; pp. 214–239.
3. Buick, R. Microfossil recognition in Archean rocks: An appraisal of spheroids and filaments from a 3500 M.Y. old chert-barite unit at North Pole, western Australia. *Palaios* **1990**, *5*, 441–459.
4. Summons, R.E.; Walter, M.R. Molecular fossils and microfossils of prokaryotes and protists from Proterozoic sediments. *Amer. J. Sci.* **1990**, *290*, 212–244.
5. Cady, S.L. Astrobiology: A new frontier for 21st century paleontologists. *Palaios* **1998**, *13*, 95–97.
6. Schopf, J.W. Fossils and Pseudofossils: Lessons from the Hunt for Early Life on Earth. In *Size Limits of Very Small Microorganisms: Proceedings of a Workshop*; National Academy Press: Washington, DC, USA, 1999; pp. 88–93.
7. Westall, F. The nature of fossil bacteria: A guide to the search for extraterrestrial life. *J. Geophys. Res.* **1999**, *104*, 16437–16451.
8. Cady, S.L. Paleobiology of the Archean. *Advan. Appl. Microbiol.* **2001**, *50*, 3–35.
9. Cady, S.L. Formation and preservation of bona fide microfossils. In *Signs of Life: A Report Based on the April 2000 Workshop on Life Detection Techniques*; The National Academies Press: Washington, DC, USA, 2002; pp. 149–155.
10. Cady, S.L.; Farmer, J.D.; Grotzinger, J.P.; Schopf, J.W.; Steele, A. Morphological biosignatures and the search for life on Mars. *Astrobiology* **2003**, *3*, 351–368.
11. Schopf, J.W. Geochemical and submicron-scale morphologic analyses of individual Precambrian microorganisms. In *Geochemical Investigations in Earth and Space Science: A Tribute to Isaac R Kaplan*; The Geochemical Society Special Publications **2004**; *9*, 365–375.
12. Schopf, J.W. Fossil evidence of archaean life. *Phil. Trans. Roy. Soc. B-Biol. Sci.* **2006**, *361*, 869–885.
13. Summons, R.E.; Albrecht, P.; McDonald, G.; Moldowan, J.M. Molecular biosignatures. *Strateg. Life Detection—Space Sci. Ser. ISSI* **2008**, *25*, 133–159.
14. Schopf, J.W.; Kudryavtsev, A.B.; Sugitani, K.; Walter, M.R. Precambrian microbe-like pseudofossils: A promising solution to the problem. *Precambrian Res.* **2010**, *179*, 191–205.

15. Summons, R.E.; Amend, J.P.; Bish, D; Buick, R.; Cody, G.D.; Des Marais, D.J.; Dromart, G.; Eigenbrode, J.L.; Knoll, A.H.; Sumner, D.Y. Preservation of martian organic and environmental records: Final report of the Mars biosignature working group. *Astrobiology* **2011**, *11*, 157–181.
16. Summons, R.E.; Hallmann, C. Organic geochemical signatures of early life on Earth. In *Treatise on Geochemistry*, 2nd ed.; Turekian, K.K., Holland, H.D., Eds.; Elsevier: Amsterdam, The Netherlands, 2014; Volume 12, Chapter 12.2, pp. 33–46.
17. Van Zuilen, M.A.; Chaussidon, M.; Rolliion-Bard, C.; Marty, B. Carbonaceous cherts of the Barberton Greenstone Belt, South Africa: Isotopic, chemical and structural characteristics of individual microstructures. *Geochimica. Cosmochimica. Acta* **2007**, *71*, 655–669.
18. Oehler, D.Z.; Robert, F.; Walter, M.R.; Sugitani, K.; Allwood, A.; Meibom, A.; Mostefaoui, S.; Selo, M.; Thomen, A.; Gibson, E.K. NanoSIMS: Insights to biogenicity and syngeneity of Archaean carbonaceous structures. *Precambrian Res.* **2009**, *173*, 70–78.
19. Lepot, K.; Williford, K.H.; Ushikubo, T.; Sugitani, K.; Mimura, K.; Spicuzza, M.J.; Valley, J.W. Texture-specific isotopic compositions in 3.4 Gyr old organic matter support selective preservation in cell-like structures. *Geochim. Cosmochim. Acta* **2013**, *112*, 66–86.
20. Van Zuilen, M.A.; Lepland, A.; Teranes, J.; Finarelli, J.; Wahlen, M.; Arrhenius, G. Graphite and carbonates in the 3.8 Ga old Isua Supracrustal Belt, southern west Greenland. *Precambrian Res.* **2003**, *126*, 331–348.
21. Holm, N.G. Serpentinization of oceanic crust and Fischer-Tropsch type synthesis of organic compounds. *Origins Life Evol. Biosphere* **1996**, *26*, 205–206.
22. McCollom, T.M.; Ritter, G.; Simoneit, B.R.T. Lipid synthesis under hydrothermal conditions by Fischer-Tropsch type reactions. *Origins Life Evol. Biosphere* **1998**, *29*, 153–166.
23. Pizzarello, S.; Shock, E. The organic composition of carbonaceous meteorites: The evolutionary story ahead of biochemistry. *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, doi:10.1101/cshperspect.a002105.
24. Eigenbrode, J.; Benning, L.G.; Maule, J.; Wainwright, N.; Steele, A.; Amundsen, H.E.; AMASE 2006 Team. A field-based cleaning protocol for sampling devices used in life-detection studies. *Astrobiology* **2009**, 9, 455–465.
25. Waldbauer, J.R.; Sherman, L.S.; Sumner, D.Y.; Summons, R.E. Late Archean molecular fossils from the transvaal supergroup record the antiquity of microbial diversity and aerobiosis. *Precambrian Res.* **2009**, *169*, 28–47.
26. Derenne, S.; Robert, F.; Skrzypczak-Bonduelle, A.; Gourier, D.; Binet, L.; Rouzaud, J.-N. Molecular evidence for life in the 3.5 billion year old Warrawoona chert. *Earth Planet. Sci. Lett.* **2008**, *272*, 476–480.
27. Marshall, C.P.; Love, G.D.; Hill, A.C.; Allwood, A.C.; Walter, M.R.; van Kranendonk, M.J.; Sowden, S.A.; Sylva, S.P.; Summons, R.E. Structural characterization of kerogen in 3.4 Ga Archaean cherts from the Pilbara Craton, western Australia. *Precambrian Res.* **2007**, *155*, 1–23.
28. Schopf, J.W.; Tripathi, A.B.; Kudryavtsev, A.B. Three-dimensional confocal optical imagery of Precambrian microscopic organisms. *Astrobiology* **2006**, *6*, 1–16.
29. Schopf, J.W.; Kudryavtsev, A.B. Confocal laser scanning microscopy and Raman imagery of ancient microscopic fossils. *Precambrian Res.* **2009**, *173*, 39–49.

30. Schopf, J.W.; Kudryavtsev, A.B. Three-dimensional Raman imagery of Precambrian microscopic organisms. *Geobiology* **2005**, *3*, 1–12.
31. Schopf, J.W.; Kudryavtsev, A.B. A renaissance in studies of ancient life. *Geol. Today* **2010**, *26*, 140–145.
32. Lepot, K.; Philippot, P.; Benzerara, K.; Wang, G-Y. Garnet-filled trails associated with carbonaceous matter mimicking microbial filaments in Archean Basalt. *Geobiology* **2009**, *7*, 393–402.
33. Schopf, J.W.; Kudryavtsev, A.B.; Czaja, A.D.; Tripathi, A.B. Evidence of Archean life: Stromatolites and microfossils. *Precambrian Res.* **2007**, *158*, 141–155.
34. Schopf, J.W.; Kudryavtsev, A.B.; Agresti, D.B.; Czaja, A.D.; Wdowiak, T.J. Raman Imagery: A new approach to assess the geochemical maturity and biogenicity of permineralized Precambrian fossils. *Astrobiology* **2005**, *5*, 333–371.
35. Beyssac, O.; Goffe, B.; Chopin, C.; Rouzaud, J.N. Raman spectra of carbonaceous material in metasediments: A new geothermometer: Raman spectroscopy of carbonaceous material. *J. Metamorph. Geol.* **2002**, *20*, 859–871.
36. Sforna, M.C.; van Zuilen, M.A.; Philippot, P. Structural characterization by Raman hyperspectral mapping of organic carbon in the 3.46 billion-year-old Apex chert, western Australia. *Geochim. Cosmochim. Acta* **2013**, *124*, 18–33.
37. Schopf, J.W.; Kudryavtsev, A.B.; Agresti, D.G.; Wdowiak, T.J.; Czaja, A.D. Laser-Raman imagery of Earth's earliest fossils. *Nature* **2002**, *416*, 73–76.
38. Cohen, P.A.; Schopf, J.W.; Butterfield, N.J.; Kudryavtsev, A.B.; Macdonald, F.A. Phosphate biomineralization in mid-Neoproterozoic protists. *Geology* **2011**, *39*, 539–542.
39. House, C.H.; Schopf, J.W.; McKeegan, K.D.; Coath, C.D.; Harrison, T.M.; Stetter, K.O. Carbon isotopic composition of individual Precambrian microfossils. *Geology* **2000**, *28*, 707–710.
40. Strauss, H.; Moore, T.B. Abundances and isotopic compositions of carbon and sulfur species in whole rock and kerogen samples. In *The Proterozoic Biosphere*; Schopf, J.W., Klein, C., Eds.; Cambridge University Press: New York, NY, USA, 1992; pp. 709–798.
41. House, C.H.; Oehler, D.Z.; Sugitani, K.; Mimura, K. Carbon isotopic analyses of *ca.* 3.0 Ga microstructures imply planktonic autotrophs inhabited Earth's early oceans. *Geology* **2013**, *41*, 651–654.
42. Oehler, D.Z.; Walsh, M.M.; Sugitani, K.; House, C.H. Spindle-Shaped Microstructures: Potential Models for Planktonic Life Forms on Other Worlds. In Proceedings of 45th Lunar and Planetary Science Conference, The Woodlands, TX, USA, 17–21 March 2014.
43. Sugitani, K.; Grey, K.; Allwood, A.; Nagaoka, T.; Mimura, K.; Minami, M.; Marshall, C.P.; van Kranendonk, M.J.; Walter, M.R. Diverse microstructures from Archaean chert from the Mount Goldsworthy—Mount Grant area, Pilbara Craton, western Australia: Microfossils, dubiofossils, or pseudofossils? *Precambrian Res.* **2007**, *158*, 228–262.
44. Oehler, D.Z.; Robert, F.; Walter, M.R.; Sugitani, K.; Meibom, A.; Mostefaoui, S.; Gibson, E.K. Diversity in the Archean biosphere: New insights from NanoSIMS. *Astrobiology* **2010**, *10*, 413–424.
45. Walsh, M.W. Microfossils and possible microfossils from the early Archean Onverwacht Group, Barberton Mountain Land, South Africa. *Precambrian Res.* **1992**, *54*, 271–293.

46. Sugitani, K.; Lepot, K.; Nagaoka, T.; Mimura, K.; Kranendonk, M.V.; Oehler, D.Z.; Walter, M.R. Biogenicity of morphologically diverse carbonaceous microstructures from the *ca.* 3400 Ma Strelley Pool Formation, in the Pilbara Craton, western Australia. *Astrobiology* **2010**, *10*, 899–920.
47. Sugitani, K.; Mimura, K.; Nagaoka, T.; Lepot, K.; Takeuchi, M. Microfossil assemblage from the 3400 Ma Strelley Pool Formation in the Pilbara Craton, western Australia: Results form a new locality. *Precambrian Res.* **2013**, *226*, 59–74.
48. Williford, K.H.; Ushikubo, T.; Schopf, J.W.; Lepot, K.; Kitajima, K.; Valley, J.W. Preservation and detection of microstructural and taxonomic correlations in the carbon isotopic compositions of individual Precambrian microfossils. *Geochim. Cosmochim. Acta* **2013**, *104*, 165–182.
49. Horita, J. Some perspectives on isotope biosignatures for early life. *Chem. Geol.* **2005**, *218*, 171–186.
50. Lollar, B.S.; Westgate, T.D.; Ward, J.A.; Slater, G.F.; Lacrampe-Couloume, G. Abiogenic formation of alkanes in the Earth's crust as a minor source for global hydrocarbon reservoirs. *Nature* **2002**, *416*, 522–524.
51. Bontognali, T.R.R.; Sessions, A.L.; Allwood, A.C.; Fischer, W.W.; Grotzinger, J.P.; Summons, R.E.; Eiler, J.M. Sulfur isotopes of organic matter preserved in 3.45-billion-year-old stromatolites reveal microbial metabolism. *Proc. Nat. Acad. Sci. USA* **2012**, *109*, 15146–15151.
52. Robert, F.; Selo, M.; Skrzypczak, A. NanoSIMS Images of Precambrian Fossil Cells. In Proceedings of 36th Lunar and Planetary Science Conference, League City, TX, USA, 14–18 March 2005.
53. Oehler, D.Z.; Robert, F.; Mostefaoui, S.; Meibom, A.; Selo, M; McKay, D.S. Chemical mapping of Proterozoic organic matter at sub-micron spatial resolution. *Astrobiology* **2006**, *6*, 838–850.
54. Kilburn, M.R.; Wacey, D. NanoSIMS analysis of Archean fossils and biomarkers. *Appl. Surf. Sci.* **2008**, *255*, 1465–1467.
55. Fletcher, I.R.; Kilburn, M.R.; Rasmussen, B. NanoSIMS m-scale *in situ* measurement of $^{13}C/^{12}C$ in early Precambrian organic matter, with permil precision. *Int. J. Mass Spectrom.* **2008**, *278*, 59–68.
56. Wacey, D. Stromatolites in the ~3400 Ma Strelley Pool Formation, western Australia: Examining biogenicity from the macro- to the nano-scale. *Astrobiology* **2010**, *10*, 381–395.
57. Wacey, D.; Gleeson, D.; Kilburn, M.R. Microbialite taphonomy and biogenicity: New insights from NanoSIMS. *Geobiology* **2010**, *8*, 403–416.
58. Wacey, D.; Kilburn, M.R.; Saunders, M.; Cliff, J.; Brasier, M.D. Microfossils of sulphur-metabolizing cells in 3.4-billion-year-old rocks of western Australia. *Nat. Geosci.* **2011**, *4*, 698–702.
59. Oehler, D.Z.; Robert, F.; Chaussidon, M.; Gibson, E.K. Bona Fide Biosignatures: Insights from Combined NanoSIMS-SIMS. In Proceedings of Goldschmidt Conference, Vancouver, British Columbia, Canada, 13–18 July 2008.
60. Moreau, J.W.; Sharp, T.G. A transmission electron microscopy study of silica and kerogen biosignatures in ~1.9 Ga Gunflint microfossils. *Astrobiology* **2004**, *4*, 196–210.
61. Wacey, D.; Menon, S.; Green, L.; Gerstmann, D.; Kong, C.; Mcloughlin, N.; Saunders, M.; Brasier, M. Taphonomy of very ancient microfossils from the ~3400 Strelley Pool Formation and ~1900 Ma Gunflint formation: New insights using a focused ion beam. *Precambrian Res.* **2012**, *220–221*, 234–250.

62. De Gregorio, B.T.; Sharp, T.G. The structure and distribution of carbon in 3.5 Ga Apex chert: Implications for the biogenicity of Earth's oldest putative microfossils. *Amer. Mineral.* **2006**, *91*, 784–789.
63. De Gregorio, B.T.; Sharp, T.G.; Flynn, G.J.; Wirick, S.; Hervig, R.L. Biogenic origin for Earth's oldest putative microfossils. *Geology* **2009**, *37*, 631–634.
64. De Gregorio, B.T.; Sharp, T.G.; Rushdi, A.I.; Simoneit, B.R.T. Bugs or gunk? Nanoscale methods for assessing the biogenicity of ancient microfossils and organic matter. In *Earliest Life on Earth: Habitats, Environments and Methods of Detection*; Golding, S.D., Glickson, M., Eds.; Springer Science, B.V.: Dordrecht, The Netherlands, 2011; pp. 239–288.
65. Hugo, R.C.; Cady, S.L. Preparation of geological and biological TEM specimens by embedding in sulfur. *Microsc. Today* **2004**, *12*, 28–30.
66. Schopf, J.W. Microfossils of the early Archean Apex chert: New evidence of the antiquity of life. *Science* **1993**, *260*, 640–646.
67. Bernard, S.; Benzerara, K.; Beyssac, O.; Brown, G.E., Jr.; Grauvogel-Stamm, L.; Duringer, P. Ultrastructural and chemical study of modern and fossil sporoderms by Scanning Transmission X-ray Microscopy (STXM). *Rev. Palaeobot. Palynol.* **2009**, *156*, 248–261
68. Recanati, A.; Bernard, S.; Germain, D.; Charbonnier, S.; Robert, R. STXM Characterization of Fossil Organic Matter from the Montceau-les-Mines Lagerstätte (France). In Proceedings of Goldschmidt Conference, Florence, Italy, 25–30 August 2013; p. 2034.
69. Bernard, S.; Horsfield, B.; Schulz, H.-M.; Wirth, R.; Schreiber, A.; Sherwood, N. Geochemical evolution of organic-rich shales with increasing maturity: A STXM and TEM study of the Posidonia Shale (Lower Toarcian, northern Germany). *Mar. Petrol. Geol.* **2012**, *31*, 70–89.
70. Lepot, K.; Benzerara, K.; Rividi, N.; Cotte, M.; Brown, G.E., Jr.; Philipot, P. Organic matter heterogeneities in 2.72 Ga stromatolites: Alteration *vs.* preservation by sulfur incorporation. *Geochim. Cosmochim. Acta* **2009**, *73*, 6579–6599.
71. Papineau, D.; de Gregorio, B.T.; Cody, G.D.; Fries, M.D.; Mojzsis, S.J.; Steele, A.; Stroud, R.M; Fogel, M.L. Ancient graphite in the Eoarchean quartz-pyroxene rock from Akilia island in southern west Greenland I: Petrographic and spectroscopic characterization. *Geochim. Cosmochim. Acta* **2010**, *74*, 5862–5883.
72. McKeegan, K.D.; Kudryavtsev, A.B.; Schopf, J.W. Raman and ion microscopic imagery of graphitic inclusions in apatite from older than 3830 Ma Akilia supracrustal rocks, west Greenland. *Geology* **2007**, *35*, 591–594.
73. Marshall, A.O.; Jehlicka, J.; Rouzaud, J.-N.; Marshall, C.P. Multiple generations of carbonaceous material deposited in Apex chert by basin-scale pervasive hydrothermal fluid flow. *Gondwana Res.* **2014**, *25*, 284–289.
74. Schiffbauer, J.D.; Xiao, S. Novel application of focused ion beam electron microscopy (FIB-EM) in preparation and analysis of microfossil ultrastructures: A new view of complexity in early eukaryotic organisms. *Palaios* **2009**, *24*, 616–626.
75. Wacey, D. Personal Communication, 2014.
76. Clemett, S.J.; Thomas-Keprta, K.L.; Nakamura-Messenger, K. The Spatial Distribution of Organic Matter and Mineralogical Relationships in Carbonaceous Chondrites. In Proceedings of 43rd Lunar and Planetary Science Conference, The Woodlands, TX, USA, 19–23 March 2012.

77. Nakamura-Messenger, K.; Messenger, S.; Keller, L.P.; Clemett, S.J.; Nguyen, A.N.; Gibson, E.K. Coordinated *in situ* Analyses of Organic Nanoglobules in the Sutter's Mill Meteorite. In Proceedings of 44th Lunar and Planetary Science Conference, The Woodlands, TX, USA, 18–22 March 2013.
78. Clemett, S.J.; Messenger, S.; Nakamura-Messenger, K.; Thomas-Keprta, K.L.; McKay, D.S. Coordinated Chemical and Isotopic Imaging of Bells (CM2) Meteorite Matrix. In Proceedings of 45th Lunar and Planetary Science Conference, The Woodlands, TX, USA, 17–21 March 2014.
79. Mahajan, T.B.; Plows, F.L.; Gillette, J.S.; Zare, R.N.; Logan, G.A. Comparison of microprobe two-step laser desorption/laser ionization mass spectrometry and gas chromatography/mass spectrometry studies of polycyclic aromatic hydrocarbons in ancient terrestrial rocks. *J. Amer. Soc. Mass Spectrom.* **2001**, *12*, 989–1001.
80. Oehler, J.H. Irreversible contamination of Precambrian kerogen by ^{14}C-labelled organic compounds. *Precambrian Res.* **1977**, *4*, 221–227.
81. Brocks, J.J.; Love, G.D.; Snape, C.E.; Logan, G.A.; Summons, R.E.; Buick, R. Release of bound aromatic hydrocarbons from late Archean and Mesoproterozoic kerogens via hydropyrolysis. *Geochim. Cosmochim. Acta* **2003**, *67*, 1521–1530.
82. French, K.L.; Hallmann, C.; Hope, J.M.; Buick, R.; Brocks, J.J.; Summons, R.E. Archean Hydrocarbon Biomarkers: Archean or Not? In Proceedings of Goldschmidt Conference, Florence, Italy, 25–30 August 2013; p. 1110.
83. Lanier, W.P. Approximate growth rates of Early Proterozoic microstromatolites as deduced by biomass productivity. *Palaios* **1986**, *1*, 525–542.
84. Knoll, A.H.; Barghoorn, E.S. Archean microfossils showing cell division from the Swaziland system of South Africa. *Science* **1977**, *198*, 396–398.
85. Oehler, D.Z. Transmission electron microscopy of organic microfossils from the late Precambrian Bitter Springs formation of Australia; Techniques and survey of preserved ultrastructure. *J. Paleontol.* **1976**, *50*, 90–106.
86. Brocks, J.J.; Logan, G.A.; Buick, R.; Summons, R.E. Archean molecular fossils and the early rise of eukaryotes. *Science* **1999**, *285*, 1033–1036.
87. Brocks, J.J. Millimeter-scale concentration gradients of hydrocarbons in Archean shales: Live-oil escape or fingerprint of contamination? *Geochim. Cosmochim. Acta* **2011**, *75*, 3196–3213.
88. Rasmussen, B.; Fletcher, I.R.; Brocks, J.J.; Kilburn, M.R. Reassessing the first appearance of eukaryotes and cyanobacteria. *Nature* **2008**, *455*, 1101–1104.
89. Javaux, E.J.; Marshall, C.P.; Bekker, A. Organic-walled microfossils in 3.2-billion-year-old shallow-marine siliciclastic deposits. *Nature* **2010**, *463*, 934–938.
90. Schopf, J.W.; Farmer, J.D.; Foster, I.S.; Kudryavtsev, A.B.; Gallardo, V.A.; Espinoza, C. Gypsum-permineralized microfossils and their relevance to the search for life on Mars. *Astrobiology* **2012**, *12*, 619–633.
91. Howard, K.T.; Bailey, M.J.; Berhanu, D.; Bland, P.A.; Cressey, G.; Howard, L.E.; Heynes, C.; Matthewman, R.; Martins, Z.; Sephton, M.A.; Stolojan, V.; Verchovsky, S. Biomass preservation in impact melt ejecta. *Nat. Geosci.* **2013**, *6*, 1018–1022.
92. Schultz, P.H.; Harris, R.S.; Clemett, S.J.; Thomas-Keprta, K.L.; Zarate, M. Preserved flora and organics in impact melt breccias. *Geology* **2014**, doi:10.1130/G3534.1.

93. Cavalazzi, B.; Westall, F.; Cady, S.L.; Barbieri, R.; Foucher, F. Potential fossil endoliths in vesicular pillow basalt, Coral Patch Seamount, eastern north Atlantic Ocean. *Astrobiology* **2011**, *11*, 619–632.
94. Bernard, S.; Benzerara, K.; Beyssac, O.; Menguy, N.; Guyot, F.; Brown, G.E., Jr.; Goffe, B. Exceptional preservation of fossil plant spores in high-pressure metamorphic rocks. *Earth Planet. Sci. Lett.* **2007**, *262*, 257–272.
95. Allen, M.; Sherwood Lollar, B.; Runnegar, B.; Oehler, D.Z.; Lyons, J.R.; Manning, C.E.; Summers, M.E. Is Mars alive? *Eos. Trans. Amer. Geophys. Union* **2006**, *87*, 433–435.