Biogeosciences Discuss.,
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Authors' response to reviewer #02 comments

Re: Interactive comment on "Technical note: An economical apparatus for the observation and harvesting of mineral precipitation experiments with light microscopy" by Chris H. Crosby and Jake V. Bailey

Anonymous Referee ##2
Received and published: 29 January 2017

This is a useful effort and it would be valuable contribution to the literature. However, some details and other additions would improve its impact for the reader.

Page 2 A reference to add to or replace the Tomson and Nancollas reference is: Morse, J. W. "Dissolution kinetics of calcium carbonate in sea water; III, a new method for the study of carbonate reaction kinetics." American Journal of Science 274.2 (1974): 97-107. Morse was the first to propose this method.

We will add this important reference to the final draft, as well as the one suggested below:

Another reference to add about precipitating a mineral within an extracellular matrix by diffusion is Hunter, G. K., et al. "Inhibition of hydroxyapatite formation in collagen gels by chondroitin sulphate." Biochemical Journal 228.2 (1985): 463-469.

For the paragraph that begins on line 20, it would be useful to break this down into at least two sentences.

Agreed – will do so in final draft.

Line 25, consider replacing "flow" with "diffusion"

Yes – good catch. Thanks.

Page 3 Line 5: It might also be worth pointing out in some more detail in your text that the Silverman article reviews many diffusion studies within different extracellular matrices with the goal of studying biomineralization.

Will add: "Silverman and Boskey utilized the DD method to compare a variety of extracellular matrices in HAP biomineralization, as relevant to our understanding of physiologic HAP precipitation affected by a variety of proteins (Silverman & Boskey, 2004.)

Line 12: Some more experimental details about the setup, for example some solution concentrations and diffusion times with your setup, would be very helpful for the reader.

Will refer reader to SI for additional info on solution concentrations and diffusion times.

To be added to SI:
Apparatus diffusion gel material and solution concentrations can be altered as required for different experiments, but the conditions under which this apparatus was developed, and those used to produce figure 1E, are as follows:

- gelatin: type A, 1 g/10mL water, pH ~4
- cation solution: 0.133 M Ca^{2+}: $CaCl_2 \bullet 2H_2O$, pH ~8
- anion solution: 0.08 M $(PO_4)^{3-}$, $NaHPO_4$, pH ~8
- anion solution: 0.027 M F^-, $KF \bullet 2H_2O$, pH ~8

Nascent precipitation observed within ~30 hours.

Line 19: The example given for dissolving gelatine is quite aggressive; contact with hot water may alter the minerals and/or their precursors generated by the experiment. The statement that other gel materials will require different treatments is quite general; perhaps a statement suggesting that extracellular matrix removal methods must also consider the stability of the precipitation products would be more helpful.

The primary intent of this manuscript is to describe a protocol and apparatus for microscopic observation of mineral precipitation over time. It is intended that experimental details will represent conditions as desired by the experimenter. The conditions under which this apparatus was developed are given in the opening note of the SI. In particular, gelatin was chosen as a diffusion gel because its gel is transparent and amenable to optical observation and imaging, and because it is soluble in hot water. For work in which a different diffusion material is to be used, if extraction of precipitates is desired it will be important to consider both the solubility requirements of the diffusion material and the stability of precipitates and precursor molecules under whichever extraction method is required.

Line 21: The discussion about sealing strategies would benefit from more details. For example, describing a strategy with which you found success would be useful.

Will add: "A great deal of trial and error went into this protocol, the results of which are incorporated into the SI step-by-step protocol. Please see SI for details of sealing method and precautions (§ 'Add small cover slip,' 'Add setup block,' 'Add ion solutions, seal side bores.')"

Line 29: Please define "thinner" for the reader, or provide example values.

Will replace beginning of sentence with "In the assembly being described, the thickness of the gel is determined by the spacer used in forming the adaptor, as shown in SI Figure A1, in which a square cover slip is used to create the adaptor inset into which the diffusion gel will be added."

Page 4 Lines 1-5: Providing the details of an experiment, such as how the ECM was set up and inserted into the cell, what its solution composition was, and what the solution compositions were for the other solutions would help the reader to begin testing this method with a system that should provide initially positive results.

Insertion of the diffusion gel is described in SI (§ 'Mix gel' and 'add gel.')
Solution details will be added to SI, as noted in above comment.

Line 11: Please explain how the needles and syringes are used in more detail. Must the user remove the source solutions and then replace them with the next solutions? Can the use of a needle and syringe change the fluid dynamics appreciably for your system?

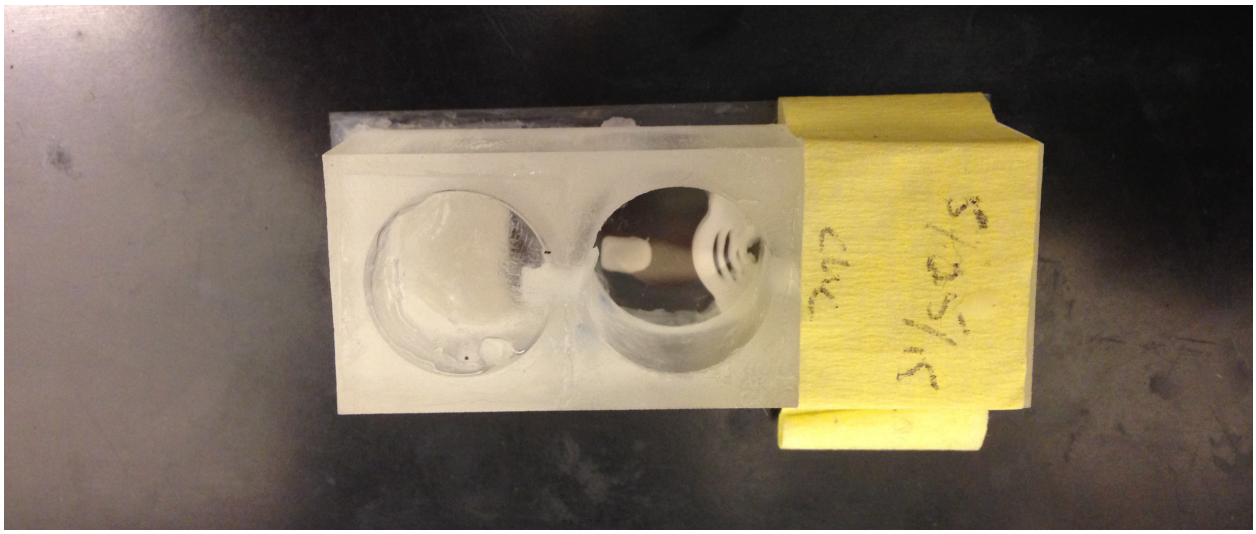
Will add descriptive text: "Addition to and/or removal and replacement initial solution can be done under sterile conditions in a biological hood as follows: Using a sterilized blade, carefully cut away the sealing material over one of the solution wells. Use a sterile needle and syringe to remove the original solution and a different sterile needle and syringe to introduce a new solution. Reseal with Tegaderm™ or similar sterile material, and seal against evaporation with clear watertight tape as described in SI § 'Add ion solutions, seal side bores'.

Figure 1d) The assembly detail could be more clear. The different stages of assembly, to the point of adding the ECM and solutions of interest, would be helpful for the reader to repeat your method.

We feel that this is well described in the assembly steps of the SI. Please indicate steps which would benefit by added description.

Figure 1e) The scale of this image is not adequate for observing the Liesegang banding easily. Consider including an inset with a higher magnification.

Final manuscript will include a higher resolution image (see image, below.)



Question: Do thinner gels heat more rapidly from the light source? Is there a method for monitoring this temperature change, and/or variation within the cell?

Will add (page 4, ~line 9) "Extra care will need to be taken when imaging the apparatus for low temperature experiments, as the gel may be heated by the light source. In this case, preliminary tests with the microscope are strongly advised to determine the degree to which this may occur, and methods for ameliorating it (such as blowing cooled air between the light source and apparatus.) Nominal monitoring of gel temperature might be possible by measuring the temperature of the two coverslips by remote sensing. Such as may be obtained by an infrared digital laser thermometer.

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