

Mar 24, 2025

Viral nanoSIMS sample preparation

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv52dp5v1b/v1>

Aditi K. Narayanan¹, Alon Philosof¹

¹California Institute of Technology

Aditi K. Narayanan: ORCID: 0000-0003-0627-1859

Alon Philosof: ORCID: 0000-0003-2684-8678

Orphan Lab



Victoria J Orphan

California Institute of Technology

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.8epv52dp5v1b/v1>

Protocol Citation: Aditi K. Narayanan, Alon Philosof 2025. Viral nanoSIMS sample preparation. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.8epv52dp5v1b/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: January 15, 2025

Last Modified: March 24, 2025

Protocol Integer ID: 118529

Keywords: viral nanosims sample preparation sample preparation nanoscale, use of viral nanosim, viral nanosim, analysis of viral suspension, interrogating marine virus, secondary ion mass spectrometry, viral suspension, marine virus, nanosim, environmental microbiology, elemental transfer with boncat, environmental sample, sample preparation, host interaction, microbiology, mass spectrometry

Funders Acknowledgements:

NOMIS Foundation

U.S. Department of Energy

Grant ID: DE-SC0020373; DE-SC0022991

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

Sample preparation nanoscale secondary ion mass spectrometry (nanoSIMS) analysis of viral suspensions. This protocol is inspired by Pasulka, A. L., Thamatrakoln, K., Kopf, S. H., Guan, Y., Poulos, B., Moradian, A., et al. (2018). Interrogating marine virus-host interactions and elemental transfer with BONCAT and nanoSIMS-based methods. *Environmental Microbiology*, 20(2), 671–692. <https://doi.org/10.1111/1462-2920.13996>. Here, we expand the use of viral nanoSIMS to make possible the analysis of non-homogenous environmental samples

Aditi K. Narayanan and Alon Philosof

- 1 Embedding of a purified viral suspension in resin for nanoSIMS analysis.

Materials

- 2
 1. Difco Agar Noble agar (BD Biosciences, Ref. 21430)
 2. Virus-free water (water filtered through Whatman Anotop 0.02µm filter, product #6809-2102)
 3. Individual 0.2mL PCR tubes (E.g. Sorenson Bioscience, Cat. 16950)
 4. 1.5mL eppendorf tubes
 5. 0.7mL tubes (E.g. Axygen Ref. MCT-060-C)
 6. Technovit 8100 embedding system (Electron Microscopy Services Cat. 14654):
Technovit 8100 infiltration solution (100mL of Technovit 8100 Basic Solution + one 0.6g package of Hardener I) and Technovit 8100 Hardener II.
 7. Microtome (e.g. the Orphan Lab has a Reichert-Jung Ultracut E Ultramicrotome)
 8. 10-well slides (e.g. Tekdon Slide ID: 10-61)
 9. SYBR Gold stain (10,000X Concentrate in DMSO), Invitrogen (now ThermoFisherScientific) Cat.#S11494
 10. VectaShield antifade mounting medium, Vector Laboratories #H-1000-10

Prepare 6% Noble agar

- 3 Measure 6g Difco Agar Noble agar (BD Biosciences, Ref. 21430).
- 4 Wash Noble agar at least three times:
 1. Add virus-free water at RT.
 2. Mix well and let settle/spin down.
 3. Decant supernatant.
 4. Repeat twice
 5. Remove as much H₂O as possible.
- 5 Final addition should be 100ml virus-free water.
- 6 Melt agar and aliquot for future use.

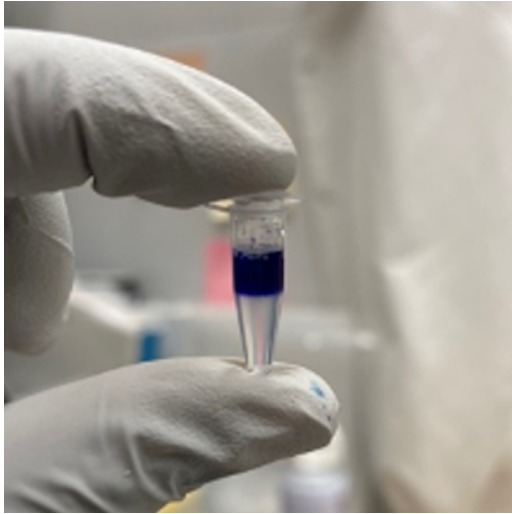


Mix Sample with Agar

- 7 Re-heat and melt 6% Noble agar aliquots if needed. We found it easiest to use a stir plate and a smaller magnetic stir bar.
- 8 Ensure the agar is not too hot. It will cook itself if allowed to heat for too long, and you will notice a change in color and smell.
- 9 Add 100 μ L of 6% Noble agar to enough PCR tubes for the number of samples/replicates you wish to embed. Allow it to harden completely

Note: This step is crucial for filling the dead volume at the bottom of the tube. VLPs are tiny, and you may not have a huge number. The conical shape of the bottom of the tube means the sample is distributed vertically. Thin sections made from the conical part contain very few VLPs.

- 10 Mix 75 μ L of sample with 5 μ L of methylene blue solution.
- 11 Add 75 μ L of your sample/methylene blue mixture on top of the 6% agar in the PCR tube
TIP: Allow the sample to come to room temperature in your hands before adding it to the PCR tube. This will help prevent fast gelling in the next step
- 12 Add 25 μ L of the warm (not too hot!) 6% agar to the 75 μ L of sample in the PCR tube. Mix very gently, avoiding bubbles. You should now have two layers in the tube: a tall cone of 100 μ L of 6% supporting agar, and a flat, broad layer of 100 μ L of 1.5% agar+sample on top.



The 1.5% sample/agar mixture in blue, and the 6% clear agar cushion below.

- 13 Allow the Sample:Agar mix to solidify fully.

Prepare Sample Plug

- 14 Carefully cut the PCR tube—one cut at the tip and another just above the plug.
- 15 Push plug into 1.5mL tube.

Dehydration of Sample Plug

- 16 Incubate 2min in each step. Fill the tube:
1. Ethanol abs 50:50 PBS.
 2. Ethanol abs 75:25 PBS.
 3. EthanolEthanol abs 90:10 PBS
 4. Ethanol abs.
 5. Ethanol abs.
- 17 1. Allow EtOEthanolH to evaporate.

TIP: It's not a big problem if the 6% cushion detaches from the 1.5% sample/agar section. You really only need the 1.5% sample block.

Infiltrate the agar plug

- 18 Add 1mL Technovit 8100 infiltration solution to the tubes containing the agar plug. Allow to sit for an hour at 4°C
- 19 Remove the infiltration solution and add another 1mL of fresh infiltration solution to each tube.
- 20 Incubate overnight at 4°C
- 21 The next day, pipette off the infiltration solution.
- 22 Put the agar plug into a new small petri dish.

Make polymerization solution and embed

- 23 Mix 500ul Hardener II + 15ml infiltration solution (make as much as needed).
- 24 Place 100μL of the polymerization solution into a 0.6mL tube
- 25 Push the agar plug into the solution such that the 1.5% sample side of the plug is facing the bottom of the tube. The goal is to have the agar completely fill the tube from wall to wall.
- 26 Use an additional 100-300μL of polymerization solution to cover the plug completely. You may need more or less depending on whether the 6% agar cushion is still attached to the sample agar.
- 27 Work quickly and avoid bubbles. The polymerization solution will start to discolor.
- 28 Allow to harden overnight. The resin block is now ready to be thin-sectioned. NanoSIMS uses sections approximately 2μm thick. We recommend anywhere from 20-30 sections per block to ensure you have reached the sample-dense section of the block.

Imaging and preparing the sample for nanoSIMS

- 29 As sections are made, transfer them into a drop of water on a 10-well slide (one thin section per well). The resin is hydrophilic and the drop of water should help it unfurl, eventually drying flat on the surface of the slide. You can see the faint iridescence where the sections are. Ideally, you will have minimal scratching and the sections will be clear and intact.



10-well slide after all water drops have dried. You can see the faint iridescence where the sections are. Ideally, you will have minimal scratching and the sections will be clear and intact, as in wells 4 and 5.

- 30 We highly recommend mapping your samples before proceeding further. Because viruses are too small to see, you do not know a) whether your section contains enough viruses and b) where in the section those viruses are located.
1. Scratch a NON-SYMMETRIC symbol (e.g. "G") into each glass well without disrupting the resin section. This will help you orient yourself during the nanoSIMS analysis, as the nanoSIMS CCD camera may transpose the image of your sample.
 2. Place a drop of 25X SYBR Gold on your samples and incubate for 30 minutes in the dark.
 3. Wick the excess stain away after 30 minutes.
 4. Allow the slide to dry completely. Then place a drop of VectaShield antifade mounting medium and mount the coverslip.
 5. Map your sections at 100X (both in the SYBR Gold channel and in brightfield), looking for edges with unusual features (like a small, distinctive tear or rippling) near high

densities of viruses. This will make it easier to find your areas of interest under the SIMS camera.

TIP: *If your microscope can resolve viruses and perform tilescons, you should easily have a reasonably high-definition map. We manually mapped our samples, and while it's doable to capture and stitch the images together by hand, we don't recommend it if you can avoid it. If you have access to a microscope like the Zeiss LSM 980 with Airyscan, you will get higher quality data that may even allow you to correlate your nanoSIMS images to your fluorescence images.*

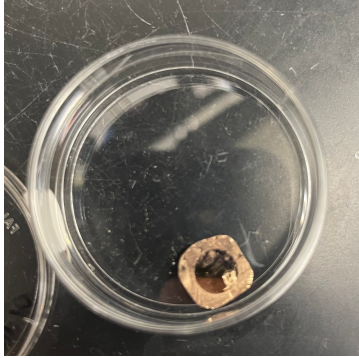
- 31 CAREFULLY wash away the coverslip mounting medium as follows
 1. Place the entire slide, including the coverslip, into a petri dish
 2. Allow DI water (or ultrapure if you prefer) to run from the tap onto the petridish; avoid placing the slide directly under the stream of water, but allow the flow of water to slowly disrupt the mounting fluid that holds the coverslip onto the slide.
 3. Gently remove the coverslip, ideally by sliding it off in the water. The tap should still be running.
 4. Continue to allow water to flow over the slide without directly placing the slide under the stream.
 5. After about 2 minutes, remove the slide and wick away excess moisture. Allow the slide to air dry.
- 32 Using a glass cutter, score and break the wells into individual pieces. You may have to use a Dremel to sand the edges so that they fit your nanoSIMS sample holder.



IMAGE CREDIT: <https://www.lss.ls.tum.de/en/boku/service/nanoscale-secondary-ion-mass-spectrometry-nanosims/service/>. We typically use the upper/left holder for our samples.

- 33 You will need to sputter coat your samples with 15nm of gold. NanoSIMS requires a conductive surface and the glass must be gold coated to create conductivity.

Naturally conductive silicon wafers are available, but may be cost prohibitive at the number of samples viral nanoSIMS requires.



Sample after cutting, sanding, and gold coating.