LSPH-NP, LSNH-NP and LSLH-NP Sulfide and its 2D imagery

The major use of sulfide probes is for measuring sulfide in sediments using LSPH-NP. The procedure for the use of soil and solution devices is similar. The sulfide accumulated on the binding layer can be measured by any conventional analytical technique for sulfide. Two-dimensional imaging is possible with a beam technique such as ICP-MS.

However, because the exposure to sulfide changes the colour of the binding layer to various shades of grey there is an easy, quick and inexpensive way to obtain 2-dimensional images of sulfide concentrations in porewater. The procedure is simple, depending only on the use of any flat bed scanner:
1. deploy probe in the sediment
2. obtain a 2-dimensional grey scale image of the binding gel with a flat bed scanner
3. calibrate the scanner using supplied colour strips
4. obtain a two dimensional image of concentration

The guidance below explains in detail how to do this. You should have calibrated data in less than half a day's work.

Deployment Scenarios

After removing the probe from its plastic bag, it should be deoxygenated by immersing it for at least two hours in a solution of 0.01 M $NaNO_3$ or NaCl bubbled quite vigorously with argon or oxygen free nitrogen. Ideally it should be inserted into the sediment without delay, but see FAQs for the need for deoxygenation. Depending on the objectives and practicalities of the study, deployment might be directly in the field or in a mesocosm or sediment core in the laboratory. In shallow water in situ deployment can be easily achieved, if necessary, with an extension to the handle of the probe, but care must be taken to avoid disturbing the sediment. For deeper water deployments, where SCUBA divers have been used, avoiding sediment disturbance is also a challenge. Whether in situ or in the laboratory, it is important to ensure that there is close contact between the face of the probe and the sediment. Silty and fine sandy sediments make deployments easy. Rougher sediments may damage the probe although various strategies, including stainless steel housing, have been used to overcome this. More details on this and general deployment strategies can be found in Ref 1.

Interpreting DGT probes supplied for measuring sulfide.

After exposure to sulfide by, for example, deployment in sediment, the binding layer of the DGT probes will become grey to black in appearance. The greyness of the binding gel can be converted to the mass of sulfide accumulated on the binding gel. This mass can be converted to a flux or interpreted as a concentration using the standard DGT equations (see FAQ: Can I calculate concentrations from deployments in soils or sediment?).

The basic procedures for doing this have been previously described in several publications, but references 2 and 3 are key. The following procedure is adapted from them.

Computer-Imaging Densitometry (CID) sounds quite complicated but it is really simple in practice. After exposure to sulfide, AgI binding gels deployed in sediments are removed from their holders and covered with a transparent plastic film (cling film) to prevent dehydration. Ensure that there are no bubbles between gel and plastic film, otherwise there may be artefacts in the scanned grey image). The gels are then scanned while wet on a flat-bed scanner (any reasonable quality scanner will do provided you keep using the same one). It is good practice to include in the same scan a gel from a DGT device that has not been deployed. The dpi resolution will determine your ultimate sulfide image resolution, a

resolution of 300 dpi corresponding to a vertical and lateral image resolution of ~0.085 mm. The grey-scale intensity of the scanned images can be determined using any appropriate software. ImageJ, a versatile, public domain, image processing and analysis software package developed at National Institutes of Health, U.S., has proved to be very effective (downloadable from http://rsb.info.nih.gov/ij/). The software allows selection of sub-areas of an image, and measurements of the grey-scale intensity of features in the image. Some notes on using ImageJ are provided at the end of this document.

Sample gels should be blank corrected by subtracting the grey-scale intensity measured on gels that have not been exposed to sulfide. The relation between blank-corrected grey-scale intensity and mass of accumulated sulfide in the AgI gels will be unique to the flatbed scanner that you have used and therefore a calibration must be performed.

Simple calibration using pre-calibrated grey-scale strips

We supply a set of grey-scale strips to enable simple calibration of the gels exposed to sulfide (available under Products and Purchases > Deployment and Calibration Systems). They have been calibrated (curtesy of Chunyang Zhou) in terms of mass of sulfide per unit area of gel by comparing their grey-scale intensity with those of standard DGT devices exposed to a set of calibration sulfide standards. The grey-scale intensities of the standards were blank corrected with reference to DGT devices from the same batch that were not exposed to sulfide. The grey-scale intensity of the supplied strips can be measured on your flat bed scanner and a calibration curve obtained. The calibration is effectively for your particular scanner and it needs to be done only once. To simplify processing we would recommend scanning the 10 calibration strips simultaneously in the same scan. The typical calibration shown was plotted in Excel using the standard procedure for fitting a trend-line using a power function.

Sensitivity range

This technique is sensitive to quite low concentrations of sulfide, but a limitation of using CID is that intense grey-scales approach black. This is why the calibration curve is curved and why it is not possible to resolve differences in concentrations for high exposures to sulfide. Note that if sulfide is measured by a standard analytical method it will increase linearly with the concentration in the exposure solution over many orders of magnitude, as for any other DGT method.

The calibration relates the grey-scale intensity to the mass of sulfide accumulated on the binding layer. As, in practice, this mass depends on the deployment time, if sulfide is very high in the sediment, short deployment times can be used to ensure that the grey-scale range is not exceeded. Conversely, long deployment times can be used to increase sensitivity when sulfide concentrations are low. The typical calibration plot shows that the measurement range is likely to be within 7 to 1200 nmol cm^{-2}. For a 24 hour deployment time, these correspond to pore water sulfide concentrations of 0.5 to 85 μmol l^{-1}.

typical sulfide calibration plot (Shuang Wu)

grey scale intensity

$y = 14.238x^{0.3404}$

sulfide nmol/cm2
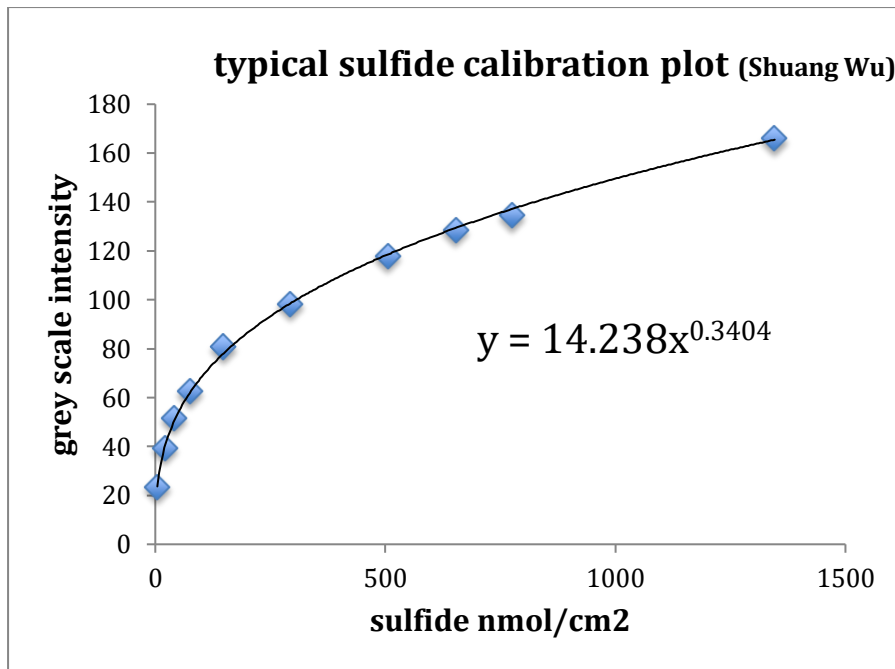
Optional calibration from solution

This section provides guidance on directly calibrating your measurements if you do not wish to use the pre-calibrated colour strips. To do this you need to deploy standard DGT devices for solutions (LSNH-NP) in known solutions containing known concentrations of sulfide to obtain binding gels with known amounts of sulfide. The calibration is effectively for your particular scanner and it needs to be done only once. After that two-dimensional images of concentrations or fluxes of sulfide can be obtained easily and quickly from each deployment of a DGT probe.

The following is a possible calibration procedure.
At least 5 solutions must be prepared containing sulfide at concentration of 10 to 1000 μmol L^{-1}. Sulfide is easily lost from solution by both oxidation and volatilization. Experience has shown (Davison, pers. comm.) that working rapidly and ensuring that the headspace in any containers is minimized can successfully minimize these effects. Using an anaerobic cabinet is not advised as the increased handling times usually increase loss by volatilization. A stock solution of sodium sulfide can be prepared by weighing a single crystal of $Na_2S.9H_2O$ and dropping it into a glass 1 L flask filled to the top with MQ-water purged with oxygen-free N_2 or Ar gas and then stoppering it without headspace. Note that crystals of $Na_2S.9H_2O$ are hydroscopic to the extent of deliquescence and so well defined crystals will not be present in old bottles of the salt. Deployment solutions of appropriate concentrations can be prepared by mounting the DGT device in a small container filled with MQ water and containing a magnetic follower. The water should be thoroughly deoxygenated with N_2 or Ar. After switching off the gas, but while stirring, an appropriate aliquot of stock solution should be added to obtain the required concentration of sulfide. To minimize losses of sulfide, the surface of the solution must then be covered, minimizing any headspace. A deployment time of 4 hours will be appropriate
The gels should be scanned in exactly the same way as the sample gels and the grey-scale intensity blank corrected as described above. The mass of accumulated sulfide per unit area of the gels (M/A) can be calculated using the following equation:
M/A = CDt/Δg
where M (μmol) is the mass of sulfide, A (cm^2) is the surface area of the exposed diffusive layer, C (μmol L^{-1}) is the sulfide concentration in the calibration solution, D (cm^2 s^{-1}) is the temperature-corrected diffusion coefficient for HS-(aq), t (s) is the deployment time, and Δg

(cm) is the thickness of the diffusion layer (diffusive gel and prefilter). This website has recommended diffusion coefficients for a range of temperatures. A nonlinear relationship between grey-scale intensity and mass of accumulated sulfide per unit area is obtained. Such plots have been fitted previously using a power function (see figure) or an equation of the form grey-scale = Aln(B x sulfide) where A and B are fitting constants.

Key references

1. D. F. Jolley, S. Mason, Y. Gao and H. Zhang, Practicalities of working with DGT (practical issues such as deployment, quality control and sensitivity, Ch. 10 in Diffusive Gradients in Thin-Films for Environmental Measurements, Ed. W. Davison, Cambridge University Press, 2016, Cambridge.

2. P. R. Teasdale, S. Hayward and W. Davison, In situ, high-resolution measurement of dissolved sulfide using diffusive gradients in thin films with computer-imaging densitometry, *Anal Chem*, **71**: (1999), 2186-2191.

3. A. Widerlund and W. Davison, Size and density distribution of sulfide-producing microniches in lake sediments. *Environ Sci Technol*, **41**:23 (2007), 8044-8049.

Suggestions for using ImageJ

Before following these instructions (supplied curtesy of Chunyang Zhou) we suggest you familiarise yourself with the basic features of the software. Various short tutorials for its use are available on YouTube, among them 'Tutorial ImageJ' and 'Optical Density Using ImageJ'.

1) Open your grey-scale image in Image J; select the part you are interested in, adjust the width and length of your image (Image > properties) and invert it (Edit > Invert).

2) Transform the image to results, saving the results in Excel (the outcome is a series of grey-scale values, as each pixel of your image has a grey-scale value) (Image > Transform > Image to results).

3) Blank correct in Excel by subtracting values obtained for your unexposed devices. Calculate the concentration values from the blank-corrected, grey-scale values in Excel, using the equation from your calibration plot (as in the figure above).

4) Import your calculated concentration values into Image J.

5) Transform your results to an image (Image > Transfrom > Results to Image). Now you have a 2D concentration plot. Image J allows you to manipulate this is many ways, for example conversion to a color plot (Image > lookup tables > spectrum) or insertion of a calibration bar (Analyze > Tools > calibration/scale bar).