

Simple protocol for using AgI DGT probes to measure sulfide in sediments

This is a simple set of instructions. Further [details on sediments deployments](#) and the basis of [sulfide determination from a greyscale image](#) are available.

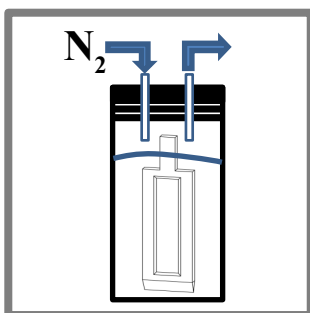
General deployment considerations

Sediments need to be sufficiently fine grained and soft to allow insertion of the probe without tearing the membrane in the exposure window. Biofouling is not a problem for deployments in sediments.

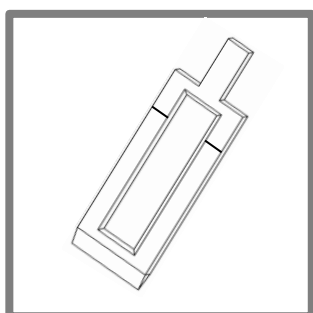
Deploying DGT probes

Deoxygenation?

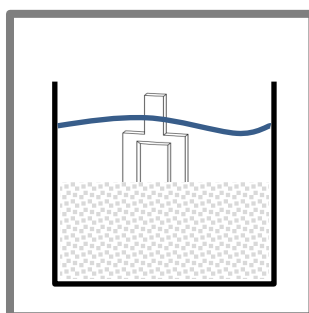
Deoxygenation (Step 1) is optional. Good results can be obtained without it. For the pros and cons of deoxygenation see either FAQs or the more detailed guide for sulfide. If you prefer to deoxygenate follow these brief instructions. Immerse the DGT probe for 24 h in a clean container filled with 0.03 M NaCl solution through which N₂ is steadily bubbled. Seal this container to maintain it oxygen-free for transportation to the deployment site (ideally no more than a few hours).



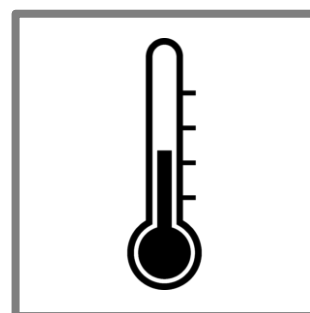
1. If probes are to be deoxygenated include this step, otherwise proceed directly to step 2. Deoxygenated probes must be deployed immediately to minimize air contact. Speed is not critical if deployment is from the plastic bag,



2. Remove the probe from either the oxygen-free container or its supplied plastic bag. Quickly mark the plastic body of the probe (permanent marker) at the intended depth for the sediment-water interface.



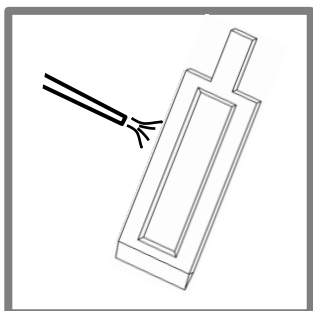
3. As soon as possible (within seconds) of removing the probe from its oxygen-free container or original bag, smoothly push it into the sediment until the mark is in line with the sediment-water interface. Keep the probe as vertical as possible during the insertion.



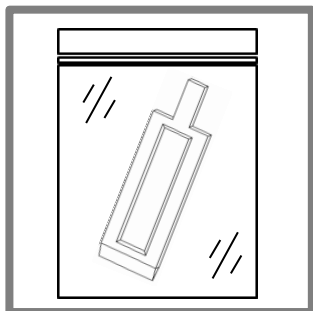
4. Record the temperature of the sediment during the deployment period. A start and end temperature will suffice. Record the deployment time to the nearest minute.

Procedure for analyzing DGT samples

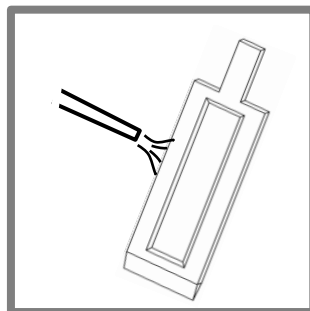
DGT Recovery and Sample Treatment



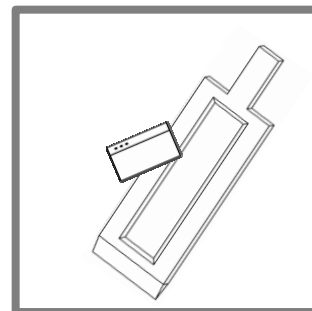
1. After retrieving the DGT probe from the sediment thoroughly rinse it with clean water.



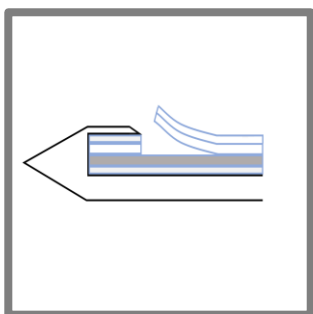
2. Place in a clean plastic bag for storage and transportation back to the lab for sample treatment.



3. Remove from the plastic bag and thoroughly rinse the probe again with clean water.



4. Either disassemble the probe or simply cut the gels and the filter membranes together along the window edges by pushing a razor blade into them in a series of guillotine-like steps.



5. Carefully lift out the four layers together. Please note that there is a backing filter membrane layer at the bottom of the AgI binding gel.



6. Place them on a supporting sheet of fairly thick polythene. Carefully peel of the top membrane filter and clear diffusive gel to reveal the underlying AgI binding gel. Its appearance from light grey-yellow to dark grey reflects the concentration of sulfide in the sediment.

Note If the binding gel appears brown, it is due to freshly precipitated iron oxide. The iron oxide can be removed without affecting any grey coloration by placing the binding gel with underlying support in a solution of 0.01M hydroxylamine hydrochloride for 3 hours. Rinse with water then proceed as given below.

Converting the grey and black appearance of the binding gel to concentration

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Scan

Cover the binding gel with a transparent plastic film (cling film) to prevent dehydration, ensuring that there are no air bubbles between the gel and the plastic film. Place the wet binding gel, complete with polythene supporting sheet, backing filter membrane and plastic film, on a flat bed scanner. Before scanning you need to add some other items to be scanned together. These are: firstly, a supplied moist binding gel that has not been exposed to sulfide; secondly, the ten calibration grey-scale strips. Scan and save the scanned image.

Obtain grey-scale intensity

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 in the Appendix to 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.

Calibrate to quantify sulfide

The supplied set of grey-scale strips enable calibration of the gels exposed to sulfide. They have been calibrated 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 calibrated sulfide standard solutions. 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 need to be measured with your scanner. Plot the grey-scale intensity values against the sulfide quantity provided (in units of nmol/cm^2) to obtain your unique calibration curve. This calibration is for your particular scanner and it needs to be done only once. It will be a curved line. If the calibration is plotted in Excel, the standard procedure for fitting a trend-line using a power function can be used to fit the curve (see Appendix).

Sensitivity range

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 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 $\mu\text{mol L}^{-1}$ (0.1 to 2.7 mg L^{-1}).

Calculate the DGT measured concentration in the sediment

The calibration curve provides the mass per unit area accumulated, M_A , in the deployment time, t (seconds). This can be used to calculate the mean concentration during the deployment time of sulfide in the pore water at the surface of the device, which we call C_{DGT} .

$$C_{\text{DGT}} = M_A \Delta_g / Dt$$

Δ_g (usually 0.094 cm) is the total thickness of the materials in the diffusion layer (diffusive gel and filter membrane).

D ($\text{cm}^2 \text{s}^{-1}$) is the diffusion coefficient of analyte in the material diffusion layer for the deployment temperature (see

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diffusion coefficients). Recommended units to facilitate easy calculation are shown.

Appendix

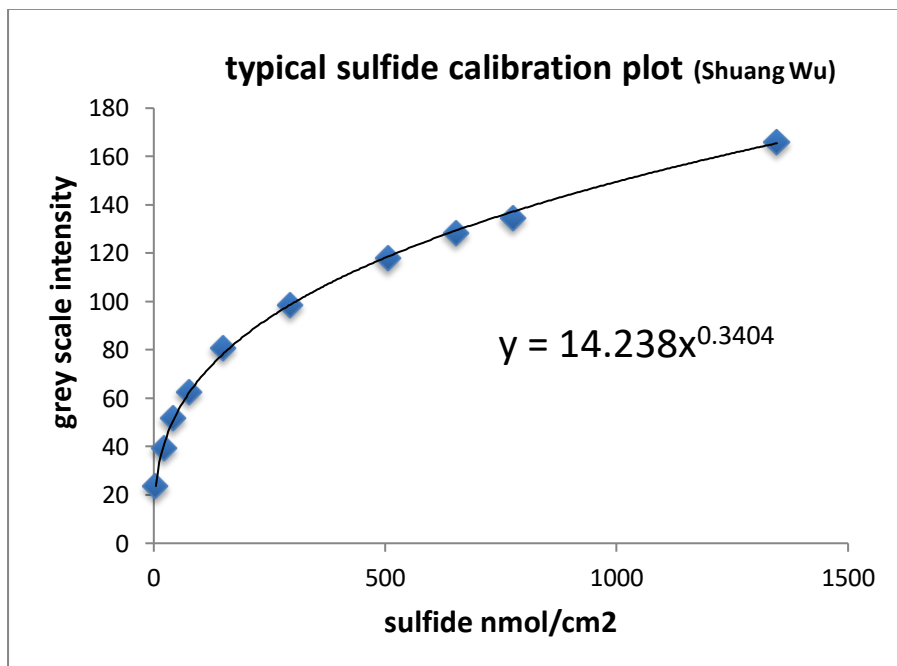
Suggestions for using ImageJ

Before following these instructions 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 > Transform > Results to Image). Now you have a 2D concentration plot. Image J allows you to manipulate this in many ways, for example conversion to a color plot (Image > lookup tables > spectrum) or insertion of a calibration bar (Analyze > Tools > calibration/scale bar).

Example calibration plot

(it is essential that you obtain your own for your chosen scanner)



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