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Optimization of colorimetric DET technique for the in situ, two-dimensional measurement of iron(II) distributions in sediment porewaters

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Abstract

The recently developed colorimetric diffusive equilibration in thin films (DET) technique for the in situ, high-resolution measurement of iron(II) in marine sediments is optimized to allow measurement of the higher iron concentrations typical of sediment porewaters. Computer imaging densitometry (CID) is used to analyze the retrieved samplers following exposure to ferrozine, a colorimetric reagent selective for iron(II). The effect of ferrozine concentration, image processing parameters and ionic strength are investigated to improve the applicability of this technique to a wider range of aquatic systems than reported in the first publications of this approach. The technique was optimized to allow detection of up to 2000 μmol L⁻¹ iron(II), a four-fold increase on the previous upper detection limit of 500 μmol L⁻¹. The CID processing of the scanned color image was also optimized to adjust the sensitivity of the assay as required; by processing the image with different color channel filters, the sensitivity of the assay can be optimized for lower concentrations (up to 100 μmol L⁻¹) or higher concentrations (up to 2000 μmol L⁻¹) of iron(II), depending on the specific site characteristics. This process does not require separate sampling probes or even separate scans of the DET gels as the color filter and grayscale conversion is done post-image capture. The optimized technique is very simple to use and provides highly representative, high-resolution (1 mm) two-dimensional distributions of iron(II) in sediment porewaters. The detection limit of the optimized technique was 4.1 ± 0.3 μmol L⁻¹ iron(II) and relative standard deviations were less than 6%.

Keywords: DET; CID; iron; biogeochemistry; ferrozine; high-resolution
1.0 Introduction

The study of sediment biogeochemistry is a challenging and complex area due to heterogeneous chemical distributions. Traditional sediment sampling techniques require the removal and processing of sediment cores [1-3]. Porewater profiles are typically obtained by slicing and centrifugation of sediment samples, resulting in large volumes of sediment porewater being mixed and thus the measured concentrations representing an average of the sampled volume [4, 5]. This process can introduce sampling errors, particularly where reactive chemical species may be present in the same sample (e.g. iron(II) and sulfide) [3-7]. Furthermore, these traditional methods of porewater analysis fail to provide data at sufficient spatial resolution to allow investigation of heterogeneous solute distributions or concentration gradients of sediment porewater solutes, which often occur on the millimeter scale [1, 2, 8]. Diffusion-based techniques, such as diffusive equilibration in thin films (DET), avoid the disadvantages of traditional sediment sampling as they measure porewater profiles in situ, thus representing the actual chemical distributions present in the sediment [1, 4]. Additionally, they allow analysis at high spatial resolution (millimeter to sub-millimeter) [9], in two-dimensions [10] and only sample a very small volume of sediment porewater; approximately 10 μL for a gel analyzed at 1 square millimeter resolution (based on a 10 mm depletion distance; see Harper et al. [11] for details), thereby allowing small-scale heterogeneity to be observed and mechanistic interactions to be assessed and interpreted [8]. The interpretation of DET measurements has been comprehensively discussed by Harper and co-workers [11] and Davison and co-workers [12] and the techniques have been utilized for the measurement of trace metals in both fresh [13, 14] and marine [15, 16] sediment porewaters.

In situ sampling is particularly important for redox-sensitive analytes such as iron, which may be present as reduced iron(II) in the sediment and is rapidly oxidized to iron(III) upon exposure to oxygen [6, 7, 17, 18]. The biogeochemical cycling of iron is of particular importance due to its role as both a source and sink for many other important solutes including phosphorus and arsenic, both of which are
present as oxyanions in water, are strongly bound to solid-phase iron oxides and are remobilized via reductive dissolution of iron(III) minerals [19-21]. Understanding the biogeochemistry of iron is integral to determining the cycling of these solutes and how other factors such as eutrophication-induced or seasonal anoxia can affect their fluxes both to and from the sediment.

A colorimetric DET technique recently developed by Jezequel and co-workers [22] and Robertson and co-workers [6] allows the two-dimensional measurement of iron(II) distributions in sediment porewaters. A colorimetric reagent, selective for iron(II), was equilibrated with a hydrogel that was then placed onto the hydrogel of a DET sampler, immediately following removal from the sediment. The colored complex that formed within the two gels was analyzed by scanning on a conventional flatbed scanner and converting the grayscale intensities to iron(II) concentrations utilizing a calibration curve [6]. This approach, known as computer imaging densitometry (CID), was first applied to environmental analysis for the measurement of dissolved sulfide [23] by a diffusive gradients in thin films (DGT) technique. This technique has been applied to the investigation of iron(II) and sulfide distributions in mesocosms containing bioturbators [24] and to study the effect of seagrass on iron(II) and sulfide distributions in natural sediments [25]. A colorimetric DET technique similar to that described in this study has also been developed for the measurement of phosphate distributions in sediment porewaters [26]. These diffusive techniques utilizing CID have proven to be useful and simple techniques for quantitative imaging of sediment solute distributions in one or two dimensions. Unfortunately, the maximum measurement range of the colorimetric DET techniques developed for iron(II) to date is $0.6 – 500 \, \mu \text{mol L}^{-1}$ [6], which is not sufficiently large to allow the measurement of the full range of iron(II) concentrations typically seen in many freshwater sediment porewaters where iron(II) is not constrained by sulfide [27]. Furthermore, the technique has not been evaluated with respect to temperature, ionic strength ranges or reagent concentration. In this study, we optimized the
colorimetric DET technique for iron(II) by modifying the concentration of colorimetric reagent and the CID parameters to allow the quantification of a much larger range of iron(II) concentrations.

2.0 Experimental

2.1 Reagents, materials and solutions

Ultra-pure deionized water (Milli-Q Element, Millipore) was used for the preparation of all solutions. Bisacrylamide-cross linked hydrogels for the DET technique were prepared as described previously [6]. Ferrous iron stock solutions were prepared by dissolving ammonium iron(II) sulfate (AR Grade, Merck) in 0.01 mol L$^{-1}$ hydroxylamine hydrochloride (ACS Grade, Alfa Aesar). All dilutions of the ferrous iron stock solution were made in 0.01 mol L$^{-1}$ hydroxylamine hydrochloride in 0.01 mol L$^{-1}$ sodium chloride (AR Grade, Merck). Hydroxylamine was added to prevent oxidation of iron(II) to iron(III) during experiments. The DET colorimetric reagent consisted of 0.01 mol L$^{-1}$ ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4’,4’’-disulfonic acid sodium salt, Fluka) in an acetate buffer consisting of 0.1 mol L$^{-1}$ acetic acid (AR Grade, Merck) and 0.1 mol L$^{-1}$ sodium acetate (AR Grade, Chem. Supply). DET sediment probe assemblies were purchased from DGT Research Ltd., washed in 10% (v/v) HNO$_3$ (AR Grade, Merck) for 24 h and then rinsed in deionized water prior to assembly as described previously [6]. An iron(II) quality control standard was prepared from a NIST-certified 1000 mg L$^{-1}$ iron standard (High Purity Standards, Charleston) reduced to iron(II) by addition of hydroxylamine hydrochloride. An aliquot of 1 mol L$^{-1}$ hydroxylamine hydrochloride was added to the 1000 mg L$^{-1}$ iron standard in a ratio of 1:10 and left to react for at least 1 h before use. This standard was then diluted to 100 µmol L$^{-1}$ (5.58 mg L$^{-1}$) in 0.01 mol L$^{-1}$ hydroxylamine hydrochloride and 0.01 mol L$^{-1}$ sodium chloride.
2.2 Computer imaging densitometry (CID)

CID analysis was done with a commercially available flatbed scanner (Canon LiDE 200) and image processing software (GIMP v2.6, available free of charge from http://www.gimp.org). Once exposed to the staining gel, the iron(II) within the DET gel reacted with the ferrozine reagent forming a magenta colored complex. To ensure minimal loss in profile fidelity during the staining procedure, it is important that the staining gel is well drained following removal from the ferrozine reagent so that iron(II) reacts with ferrozine within the gel structure, rather than with excess ferrozine reagent present on the surface of the gel. The optimized staining time for this technique was 10 minutes (Section 3.2).

The stained gels were scanned in color at 300 dpi resolution, saved as a TIFF file and then processed in GIMP. Processing of the images involved conversion to grayscale so that color variation was eliminated and the grayscale intensity of the image was the only variable being measured. The channel mixer function in GIMP can be used to convert images to grayscale using a red, green or blue color filter, or a combination thereof. The optimization of this step is discussed in Section 3.1. Following the selection of an appropriate color filter, the image was converted into grayscale, resized so that one pixel equaled one square millimeter and saved as a TIFF image file. The maximum resolution of this technique (one square millimeter) is limited by the thickness of the diffusive layer; the use of thinner diffusive gels will permit analysis at higher resolution [28]. Grayscale images were imported into ImageJ (available free of charge from http://rsbweb.nih.gov/ij/), where grayscale values were measured and exported to Microsoft Excel. Blank gels were analysed in the same way and blank grayscale values were subtracted from the sample grayscale values prior to calculation of concentration.

2.3 Ferrozine concentration, color channel and staining time

The method was optimized by varying two important parameters: the concentration of ferrozine in the staining gel and the color channel used for grayscale conversion. The technique described by Robertson and co-workers [6] relied on the reaction of iron(II) (that is equilibrated within the retrieved
bisacrylamide hydrogel sampling gel) with a second hydrogel equilibrated with 0.001 mol L$^{-1}$ ferrozine in 0.1 mol L$^{-1}$ acetic acid (staining gel), which was overlain onto the DET sampling gel immediately after retrieval. The iron(II) diffuses from the retrieved gel into the staining gel, forming a magenta colored complex with ferrozine in a 3:1 molar ratio (ferrozine: iron) [29]. Robertson and co-workers [6] reported an upper measurement limit of 500 µmol L$^{-1}$ for the technique. In the present study we increased the ferrozine concentration ten-fold to 0.01 mol L$^{-1}$ and evaluated this against the procedure used by Robertson and co-workers [6].

Calibration standards were prepared at 0, 10, 20, 50, 75, 100, 200, 500, 1000, 2000 µmol L$^{-1}$ in 0.01 mol L$^{-1}$ sodium chloride and 0.01 mol L$^{-1}$ hydroxylamine hydrochloride, to prevent oxidation of iron(II). Gel discs (n=3 for each calibration standard) were equilibrated in the calibration solutions for at least 1 h, removed and placed on a ferrozine staining gel and covered with a clear acetate transparency sheet. The calibration curves were scanned at 5, 10, 15 and 20 minutes, and processed as described in Section 2.2.

To determine the optimal color channel for grayscale conversion of the acquired image, calibration standards were analyzed and converted to grayscale utilizing either a red, green, or blue color channel filter. The selection of the best color channel filter was based on the requirements of sensitivity and upper measurement limits.

2.4 Effect of ionic strength

Gel discs were equilibrated with 100 or 1000 µmol L$^{-1}$ iron(II) prepared in 0.01 mol L$^{-1}$ hydroxylamine hydrochloride and 0.01, 0.1 or 0.7 mol L$^{-1}$ sodium chloride. The gels were then stained and scanned as described in Section 2.2.
2.5 Deployment in a freshwater sediment mesocosm

Freshwater sediment was collected from the Coomera River on the Gold Coast, Queensland, Australia, sieved to 1 mm to remove biota and large debris and 13 L placed into a plastic mesocosm (30 cm Ø, 30 cm height) with 7 L of overlying water collected from the same site. The mesocosm was allowed to stabilize for ~3 months prior to deployment. A DET sediment probe with a 0.8 mm bisacrylamide hydrogel was deployed in the mesocosm for 24 h. Upon retrieval the probe was briefly rinsed with deionized water and the bisacrylamide hydrogel cut out of the front window using a razor blade. This gel was then analyzed for iron(II) as per Section 2.2. The time between removal and staining of the probe was less than 1 minute to minimize the oxidation of iron(II) in air.

3.0 Results and Discussion

3.1 Ferrozine concentration and color channel

The existing colorimetric iron(II) DET technique has an upper measurement range of 500 µmol L\(^{-1}\). We propose this could be due to an insufficient concentration of ferrozine reagent (0.001 mol L\(^{-1}\)), which is required in a 3:1 ratio of ferrozine to iron [29]. Assumining that all iron(II) in the sample gel reacted with all ferrozine in the staining gel, a concentration of 0.001 mol L\(^{-1}\) ferrozine would limit measurement to ~330 µmol L\(^{-1}\) iron(II). By increasing the concentration of ferrozine reagent to 0.01 mol L\(^{-1}\), higher concentrations of iron(II) should theoretically be measureable. Figure 1 shows the results of calibration curves made using 0.01 mol L\(^{-1}\) ferrozine reagent for a concentration range up to 2000 µmol L\(^{-1}\) iron(II) with a staining time of 10 minutes (see Section 3.2).

The original colorimetric iron(II) DET technique utilized a green channel filter during grayscale conversion to maximize the sensitivity of the measurement. While this does improve the sensitivity of
the technique, it also means the maximum grayscale values are reached earlier in the concentration range. In this case, grayscale intensity plateaus at iron(II) concentrations >500 μmol L⁻¹ for the calibration curve processed with a green filter. By utilizing a different color channel filter, it is possible to adjust the sensitivity to allow higher concentrations of iron(II) to be measured. The red channel filter results in a calibration curve that can be used to measure concentrations as high as 2000 μmol L⁻¹ iron(II) with an acceptable degree of accuracy. Although this approach results in some sensitivity loss at the low end of the concentration range (see Table 1 for detection limits), this is not expected to be an issue for most deployments. Processing the calibration data with a blue channel filter resulted in a very similar result to the red channel filter, but with linearity to 100 μmol L⁻¹ opposed to 200 μmol L⁻¹ for the red channel. Therefore, the blue channel calibration was not investigated any further.

The reason for the different sensitivities when using the different color channel filters is related to the magenta color of the ferrozine complex. Magenta is composed primarily of the blue and red wavelengths of light, with a relatively small proportion of green wavelengths. When a single color is converted to grayscale, the more intense the color, the higher the grayscale value it will have, resulting in grayscale intensity closer to white. When applying a green filter, the red and blue wavelengths that form the majority of the magenta color do not contribute to the grayscale intensity, so the images are darker (not as close to white) than if the red or blue wavelengths were included. This means that the grayscale values approaches the black end of the spectrum over a smaller range of color intensities (iron(II) concentrations), which increases sensitivity but limits the maximum measurement range. When a red filter is used, the red wavelengths of light, which form a larger proportion of the magenta color than green wavelengths, contribute to the grayscale value so that black is approached over a larger range of color intensities (iron(II) concentrations), effectively increasing the measurement range but decreasing the sensitivity.
In order to use Figure 1 to calibrate the technique, a curve needed to be fitted to the data. A logarithmic function fitted the majority of the data well but due to the large range of concentrations, did not provide an accurate fit for the lower concentrations. Therefore, a new approach for calibration was taken, whereby the data was split into two separate calibration curves (Figure 2).

The grayscale intensity followed a linear relationship up to 200 \( \mu\text{mol L}^{-1} \) iron(II), after which point a logarithmic relationship fitted the data up to the maximum of 2000 \( \mu\text{mol L}^{-1} \) iron(II). This split calibration curve allows a more accurate calibration to be performed compared to trying to fit a single curve to the entire data set. For blank-corrected grayscale intensity values of \( \leq 46 \) the linear calibration should be used, whereas for grayscale intensity values above this the logarithmic calibration should be used.

The two calibration curves meet at the 200 \( \mu\text{mol L}^{-1} \) calibration point, corresponding to a grayscale intensity of 45.8. To determine how well the calibration curves agree at this point, the predicted concentration for this grayscale intensity value was calculated from both calibration curve equations. The linear calibration curve predicted an iron(II) concentration of 197.8 \( \mu\text{mol L}^{-1} \) and the logarithmic calibration curve predicted an iron(II) concentration of 201.1 \( \mu\text{mol L}^{-1} \) for the same grayscale value. This is a difference of approximately 2\%, indicating that both calibration curves accurately predict the iron(II) concentration at the crossover point of the calibration range, and thus that both calibration curves can be used in conjunction to measure a wide range of iron(II) concentrations.

By processing the calibration curve using a green channel filter, as described by Robertson and co-workers [6], the sensitivity of the measurement is increased. If concentrations of iron(II) are \(< 100 \mu\text{mol} \)
(corresponding to grayscale intensity on the red channel image of <24) the image can be reprocessed to grayscale using the green channel filter and the green channel calibration curve applied (Figure 3). The higher sensitivity of the green channel is evident from the slope of the regression line for the green channel filter calibration, which is more than three-fold higher than the linear calibration curve obtained using the red channel filter. The ability to adjust the sensitivity of the measurement by simply reprocessing the image with a different color channel filter results in a versatile and useful measurement technique that can be adapted to a wide range of concentrations.

3.2 Staining time and temperature

The effect of staining time was evaluated by scanning the calibration curves at five-minute intervals for 30 minutes. For concentrations less than 200 μmol L⁻¹ the grayscale values of the red color channel calibration curve did not change after 10 minutes of staining time, indicating that the color reaction was complete by this stage. For concentrations higher than 200 μmol L⁻¹ the color reaction took up to 25 minutes to go to completion, as indicated by no further change in grayscale intensity. However, as long as the staining time is controlled accurately during the analysis, the color reaction is not required to be complete to allow quantitative measurement of iron(II). After 10 minutes of staining time, the grayscale intensity of the highest calibration standard is ~80% of the value after 25 minutes. Therefore, given that diffusive relaxation will increase over time thereby decreasing the fidelity of the measured iron(II) distributions (see Harper et al. [11] for details), a 10 minute staining time was chosen to give the best compromise between color development and minimizing diffusive relaxation of the iron(II) distribution. Similar results were observed for the green color channel calibration curve, with no further color development after 10 minutes of staining time, indicating that 10 minutes is an appropriate staining time to use regardless of the calibration curve utilized. This differs from Robertson and co-workers [6], who found an optimal staining time of 15 minutes, probably due to the higher
concentration of ferrozine reagent used in this study that ensured rapid reaction times and a sufficient excess of the ferrozine reagent.

During the initial development of this optimized method the effect of temperature on color development was evaluated by preparing, staining and analyzing calibration curves at 15, 25 and 35°C. This range of temperatures had a negligible effect on color development, with the slopes of the linear calibration curves from 0 – 200 μmol L⁻¹ agreeing to within 3%. For analysis temperatures outside of the tested range the authors recommend performing a field calibration as described in Section 3.4.

3.3 Quality control, method detection limits and reproducibility

Method detection limits (3σ) were calculated based on the standard deviation of grayscale values from blank gels analyzed at 1 mm² = 1 pixel resolution (n=100 pixels). Mean values were obtained by replicating this analysis three times. These method detection limits are higher than those reported by Robertson and co-workers (0.6 μmol L⁻¹) [6] due to a more representative MDL calculation being used in this study. Robertson and co-workers calculated their MDL based on the variation between three gels that were stained, scanned and then their grayscale intensity values averaged over the area of the gel, effectively eliminating the variation in grayscale intensity values on the single pixel scale. This technique is most commonly used on the single pixel scale to obtain high-resolution measurements, therefore, the MDL should represent this by taking the variation of each pixel into account, as the authors have done in this case.

A 100 μmol L⁻¹ quality control standard prepared from a separate, NIST-certified 1000 mg L⁻¹ iron standard solution was also analysed to investigate the accuracy and reproducibility of the technique (Table 1).
The QC recovery is an average from four gels, with the error of the mean representing the standard deviation between gels. The reproducibility, however, represents the mean variation of grayscale intensity within each QC gel analyzed at $1 \text{ mm}^2 = 1 \text{ pixel}$ resolution, which better represents the reproducibility of the technique when deployed \textit{in situ} and analyzed as a high-resolution, two-dimensional technique. Reproducibility was excellent both between and within scanned gels. The QC recoveries indicate that both approaches are capable of accurately measuring iron(II) concentrations.

The MDL is higher when processing and analyzing the data based on the red channel calibration compared to the green channel calibration, which is to be expected based on the different sensitivities of the two methods. These detection limits are sufficiently low to allow analysis of a wide range of iron(II) concentrations in sediment porewaters, especially considering the representative nature of the DET measurement [1, 4] and the high-resolution, two-dimensional capabilities. The reproducibility is also higher when using the green channel calibration, although both are sufficiently low to ensure precise results. Additionally, since data from the same scanned image can be analysed using both color filter channels, the image or specific parts of the image can be analysed using the calibration most appropriate to the iron(II) concentration range present.

The interference of the colorimetric measurement of ferrous iron by major inorganic ions has been previously investigated by Stookey [29]. They found that of the major ions tested, only divalent cobalt and monovalent copper caused a minor positive interference; copper present at five times the ferrous iron concentration resulted in a 15% overestimation and cobalt present at eight times the ferrous iron concentration resulted in a 5% overestimation [29]. This minor positive interference will be negligible in sediment porewater where iron concentrations will typically be much higher than most other metal ions.
3.4 Effect of ionic strength

The effect of ionic strength on the colorimetric DET technique was evaluated by analysis of hydrogel discs equilibrated in 100 or 1000 µmol L\(^{-1}\) iron(II) standard solutions prepared at 0.01, 0.1 and 0.7 mol L\(^{-1}\) sodium chloride (Table 2).

A minor effect of ionic strength is observed, which is more pronounced at 100 µmol L\(^{-1}\) iron(II) than at 1000 µmol L\(^{-1}\) iron(II). The difference between the highest and lowest recoveries is 13% and 6% for the 100 and 1000 µmol L\(^{-1}\) iron(II) standards, respectively. This difference is low and so calibrations at individual ionic strengths are unnecessary. However, given the ease-of-use of this technique, calibrations curves could be analyzed in the field alongside samples at matrix-matched ionic strengths for improved accuracy. This would involve the preparation of stable iron(II) standard solutions (prepared in a reducing agent such as hydroxylamine hydrochloride) in which polyacrylamide hydrogels are equilibrated; these could then be removed, stained and analysed alongside retrieved gel probes on-site. The effect of matrix pH on this technique is not a factor requiring optimization due to the acetate buffer used in the analysis and the wide pH working range of the ferrozine reagent (pH 4 – 9) [29].

3.5 Sediment deployment of optimized technique

To demonstrate the effectiveness of the optimized technique it was used to obtain an iron(II) porewater distribution in a freshwater sediment mesocosm (Figure 4).

The results show iron(II) first appearing at 20 mm depth, followed by an increase over the following 100 mm of the profile. The concentration reaches a maximum of 720 µmol L\(^{-1}\) at a depth of 120 mm, which is above the maximum measurement range of the original ferrozine DET technique but within
the range of the optimized technique described in this study. This demonstrates the advantage of the
optimized technique, as it is able to measure the higher iron(II) concentrations typical of freshwater
sediments. The two-dimensional distribution provides a higher degree of representativeness compared
to the averaged depth profile, showing the lateral and vertical variation in iron(II) porewater
concentration. In this case, the variation is low due to the homogenization of the sediment prior to
establishment of the mesocosm. However, in natural sediments colonized by infauna or rooted plants
porewater solute concentrations can exhibit a very high degree of lateral variation \[6, 24, 26, 30\]
making the use of methods such as the described technique for iron(II), which are able to measure
solute distributions in two-dimensions, essential.

4.0 Conclusions

The optimized colorimetric DET technique for the measurement of porewater iron(II) described in this
study provides a simple and effective method of investigating iron(II) biogeochemistry. The expanded
measurement range allows this technique to be used in freshwater sediment where iron(II)
concentrations are typically much higher than in marine sediments. Furthermore, this technique has
numerous potential applications when coupled with the diffusive gradients in thin films (DGT) or
complimentary colorimetric DET techniques for determining co-distributions of porewater solutes at
the same location in the sediment in either one or two dimensions \[6, 24\]. The representativeness, ease-
of-use and rapid data acquisition makes this optimized technique an important tool for the analysis of
iron(II) biogeochemistry and the interpretation of analytes with closely linked biogeochemical cycles.
5.0 References


Table Captions

Table 1. Method detection limits, quality control recoveries and reproducibility of the optimized colorimetric iron(II) DET technique. Values are means ± 1 standard deviation (n=3-4).

Table 2. Recoveries (%) of iron(II) measured using the optimized colorimetric DET technique at various ionic strengths. Values are means ± 1 standard deviation (n=3).
Figure Captions

Figure 1. Calibration curve for colorimetric iron(II) DET technique with 0.01 mol L$^{-1}$ ferrozine, based on grayscale conversion using a green (▲), blue (■) or red (●) color filter. Error bars represent 1 standard deviation of the mean (n=3).

Figure 2. Split calibration curve for colorimetric iron(II) DET technique showing linear relationship for lower concentrations (bottom) and logarithmic relationship for higher concentrations (top). Converted to grayscale with red color channel filter. Error bars represent 1 standard deviation of the mean (n=3). Staining time was 10 minutes.

Figure 3. Calibration curve for colorimetric iron(II) DET technique based on grayscale conversion with green color channel filter. Error bars represent 1 standard deviation of the mean (n=3).

Figure 4. One-dimensional depth profile and two-dimensional distribution of porewater iron(II) concentration in a homogenized freshwater sediment mesocosm at 1 mm resolution. Values of the one-dimensional depth profile are means of 13 individual horizontal measurements at 1 mm resolution and error bars represent plus or minus one standard deviation.