The authors would like to thank Professor Mario Giordano and Anonymous Referee #2 for their detailed and positive reviews of the manuscript and for highlighting areas requiring clarification.

M. Giordano (Referee)

- The ms by Sackett et al. represents a substantial methodological step forward in the comprehension of the species-specific responses of marine phytoplankton.
- I find this ms a very interesting and easy read. I would have liked to do these experiment myself! I strongly recommend that it is published, with only minor changes.

Points requiring clarification

Page, line	Comment	Response
	With a little more courage, the authors could have also addressed the problem of individual heterogeneity. That could be the next thing to do!	We agree that the issue of heterogeneity across the cell deserves further attention, however, this was beyond the scope of the study. Here we focus on obtaining high quality spectra from individual cells in order to assess intrapopulation variability. We will address heterogeneity across the cell in future work.
7331, 24	The background information are slightly incomplete. For instance, Marchetti et al. 2010 L&O already used FTIR spectroscopy (although not on single cells) to investigate Fe and Si response of southern ocean phytoplankton. That paper needs to be cited.	Added reference to Marchetti et al. (2010).
7331, 27	Also when referring to taxonomic application of FTIR the authors failed to cite the only two papers that actually use eukaryotic micro algae (I apologize if they happen to be mine, but such is life: Domenighni & Giordano 2009 J. Phycol; Giordano et al. Plant Ecology and Diversity 2 (2): 155-164. DOI: 10.1080/17550870903353088).	Apologies for the omission! Added reference to Domenighini & Giordano (2009) and Giordano et al. (2009).
7342, 27	To play the devil's advocate, I would say that before SR-FTIR becomes a common techniques lots more synchrotrons need to be made available. At this stage the broad use of this	This is a valid point. To clarify we have added the following text: "It is likely that the broad use of the approach outlined in this paper would be limited by access to synchrotron facilities. However, burgeoning new technology in laboratory-grade

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	approach is more a wish than reality. Perhaps the enthusiasm of the final paragraph should be somewhat mitigated.	instruments will soon make it possible to conduct measurements in single microalgal cells without the need for a synchrotron light source. For example, the newest focal plane array detectors have a higher magnification which allows infrared images to approach the spatial resolution possible using single-point mapping with an infrared microscope. Further, the advent of new extremely brilliant laboratory sources of IR light such as the quantum cascade laser are likely to further reduce the need for synchrotron sources in the future (Brandstetter et al. 2010)"

Anonymous Referee #2

- This study presents a unique dataset and therefore is of high novelty.
- The data appear to be analyzed with skill and rigor, and the resulting statistical analyses appear appropriate and robust.

Points requiring clarification

Page, line	Comment	Response
7332, 24	I am concerned about preservation of the samples prior to analysis. The phytoplankton samples were preserved with 1-2% formaldehyde and stored in the dark for 18 months until analysis. ▪ It is important that the authors demonstrate that treatment with formaldehyde does not interfere with or alter the chemical composition of the target cells. ▪ Additionally, it seems likely to me that some molecular changes occurred during the >year storage period at room temperature.	Referee #2 has raised a valid concern, we address the concern below in two parts followed by the text we have added to the manuscript. *Storage of formaldehyde-fixed samples* Limited information is available regarding the release of organic carbon molecules or elements from diatoms stored in formalin for extended periods. We plan to assess this periodically using the KEOPS2 samples and FTIR spectroscopy. However, 2% formalin (used in this study) has been determined to be the optimal concentration for minimizing changes to cell morphology while preventing microbial

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	Please discuss sample preservation and provide additional evidence and/or references demonstrating that these concerns are unfounded.	contamination (Mukherjee et al., 2014). Indeed, there are reports in the literature of formalin effectively preserving biological tissue for years (Bucklin & Allen, 2004). Importantly, sample preservation in our study was evidenced by observations that spectra from formaldehyde fixed samples did not show any pronounced differences from those observed previously from fresh microalgal samples. Moreover, there was no spectroscopic evidence of degradation products, indicating that our samples were still well preserved and not appreciably degraded. *Effects of sample treatment with formaldehyde* There has been a plethora of reports published in the biospectroscopic literature showing that subtle differences such as tissue type or disease state identification is possible using formalin fixed tissues, suggesting important spectroscopic information related to biological identity is maintained after fixation. Further, there have been a number of studies specifically examining this question (e.g. Faoláin et al. *Vib. Spectrosc.*, 2005, **38**, 121-127; Hastings et al. *Biopolymers*, 2008, **89**, 921-30; Pereira et al. *Spectroscopy: An International Journal* 2012, **27**, 399-402). All these studies showed only very subtle differences between the IR spectra of fresh and fixed tissue with the main changes being slight changes in the amide I band profile indicating changes to protein secondary structure related to the cross linking of proteins. There was little evidence of changes to other bands related to non-protein macromolecular components. Moreover, the relative intensity of bands including those from proteins were little changed meaning that estimates of macromolecular ratios, for example,

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		inferred from the spectra, were likely to be similar between fresh and fixed tissue. Last, there was no evidence of bands resulting directly from formalin in tissues that had been washed in isotonic saline solutions in the manner performed in our experiment. We have added the text "Comparison of FTIR spectra from fixed samples with those observed before from fresh microalgal samples did not show any pronounced differences."
7335, 10	The authors state that the multivariate model enables for normalization of sample thickness, but I could not find details for this in the paper. Working in transmission mode, the signals will be dependent on the thickness of the cells at the spot analyzed. Without normalizing for this, it isn't possible to separate differences in cellular concentrations vs. cell thickness between cells. ▪ How was normalization for cell thickness accomplished?	Reference (Heraud et al. 2005) added to methods to support statement that the approach accounts for differences in samples thickness. Added text "(to account for differences in samples thickness, minimise baseline differences and aid visual interpretation of spectra)"
	Cells were analyzed with a 5um beam, and only the center of each cell was analyzed (not the edges). This is important to keep in mind with diatoms given that the frustule is under-represented in the center of the cell compared to the edges.	We agree with Referee #2 that the frustule is under-represented in the centre of the cell. However, measurements taken along the edges of the cell would over-estimate frustule contributions! Our assessment is that either approach has limitations, so the best approach is to be consistent. Given that preliminary measurements from close to the edges of cells were associated with pronounced light scattering, we chose to consistently measure the cell interior.
Table 3	Please provide a table with the number of cells analyzed for each taxa from each station. It is stated that 20-50 cells were analyzed but it is not clear how many were analyzed for each taxa from each station. This information could be added to Table 3.	Data inserted into Table 3.

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7336, 20	Here and throughout the ms, the authors refer to the Si-O peak as representing silicate. However most of the Si in diatoms occurs as solid silica, although there may be some silicate (perhaps more precisely described as silicic acid) in a silica deposition vesicle. Please clarify which Si species is being detected by the technique and use the appropriate term.	We agree with the comment that the Si species being detected needs clarification. FTIR spectroscopy is sensitive to the Si-O functional groups of both crystalline silicate and silicic acid. As such we have modified the text throughout to say "silicate/silicic acid".
7337, 5	Following the general comment above, do Fragilariopsis have the highest concentrations of phosphorylate compounds, or are they just the thickest? This needs to be clarified in the methods.	Normalisation for sample thickness was clarified above (page 7335, line 10).
7337, 28	The clustering in the pooled taxa scores plot (Fig. 5b) is not apparent to me	We agree with the referee and have modified the text to say "clustering was weak in the pooled taxa scores plot".
7338, 7	This sentence ("Models that can robustly. . .") seems circular to me: if a model can discriminate between spectra from different stations, won't it also indicate that these spectra are different? Please clarify/re-write.	We have modified the text to say "The degree to which models can discriminate between cell spectra from different stations is indicative of the magnitude of change in macromolecular composition of those cells…".
7338, 16	Where is this observation about intraspecific variability in Fragilariopsis shown and described in the results?	Inserted "(Figure 5e)" at end of sentence.
7339, 23	What were silicate concentrations in the water at these stations? Was this nutrient not limiting, as inferred here? This information could be included in Table 1. This section is also an example of where the quantitative abilities of the technique need to be demonstrated earlier in order to support this discussion.	Appropriate data added to Table 1.
7340, 8	I suggest saying that "The up-regulation of these proteins would be consistent with observations. . .", as the current wording makes it sound like these proteins were actually measured in the current study.	Changed to "…would be consistent…".
7340, 16	All of these samples are from KEOPS2, correct? If so, I suggest deleting this phrase here, as it makes it some like	Phrase "In the KEOPS2 samples," deleted.

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	they are from a different group of samples than the SR-FTIR ones.	
7341, 2	"could account for increased concentrations" or "could be caused by increased concentrations"? I think it is the latter.	Changed to "…which could have led to increased concentrations…".
7341, 14	It is problematic to consider the effect of sampling time for only this station. If this is to be considered, it should be added for all stations. When were the others sampled? Can the effects of time and station be pulled apart?	Inserted "(whereas the other three stations were sampled between 12:00 and 17:30)"
7341, 23	Delete the third word ("and"). More importantly, this first sentence would be much more strongly supported if more evidence was given for the quantitative nature of SR-FTIR. Although the Beers-Lambert law is discussed in the methods, no evidence is given for the actual quantitative abilities of the technique. Thus, I question the ability of SR-FTIR to quantify nutrient status, much less growth rate, of these target cells. Please add supporting information to buttress this claim.	Deleted the word "and". Support for quantitative abilities of technique added previously to page 7335, line 10.