FTIR analysis

## Introduction

# Loading libraries and data  
library(tidyverse)  
library(patchwork)  
library(here)  
ftir\_data <- read\_csv(here("05-results/ftir-data\_long.csv"))  
grind\_data <- read\_csv(here("05-results/grind-data\_cleaned.csv"))  
ftir\_metadata <- read\_tsv(here("01-documentation/ftir-metadata.tsv"))

To determine the mineral composition and crystallisation of the artificial dental calculus samples, we used Fourier Transform Infrared (FTIR) spectroscopy. We compared the spectra of artificial dental calculus with spectra of archaeological dental calculus, as well as splitting factors.

### Materials

comb\_samples <- ftir\_metadata %>%  
 #filter(str\_detect(sample, "\\+")) %>%  
 separate\_rows(sample, sep = "\\+") %>%  
 mutate(  
 sample\_name = str\_extract(sample, "F[0-9]+.[0-9A-D0-6]+"),  
 sample = case\_when(  
 is.na(sample\_name) ~ paste0("F", day, ".", sample),  
 TRUE ~ sample  
 ),  
 sample\_name = str\_extract(sample, "F[0-9]+.[0-9A-D0-6]+")  
 )  
  
comb\_samples %>%  
 filter(source == "Artificial") %>%  
 group\_by(day) %>%   
 summarise(  
 n = n()  
 ) %>%  
 knitr::kable()

| day | n |
| --- | --- |
| 7 | 4 |
| 12 | 6 |
| 16 | 28 |
| 20 | 27 |
| 24 | 33 |

23 model biofilm samples from days 7, 16, 20, and 24, were analysed. Reference samples for comparison included two modern calculus samples and an archaeological calculus sample. The archaeological sample was an isolated tooth from Middenbeemster, a rural Dutch site from the 19th century. Samples , had to be combined to provide enough material for analysis. Only samples collected on the same day were combined with each other. Samples from days 7 and 12 did not produce enough material for a grind curve. They were largely composed of organics and proteins, I would not be able to produce a splitting factor, anyway.

grind\_data %>%  
 filter(Sample == "Artificial calculus") %>%  
 group\_by(day) %>%   
 summarise(  
 n = n()  
 ) %>%  
 knitr::kable()

Summary of samples used in grinding curves.

| day | n |
| --- | --- |
| 16 | 16 |
| 20 | 15 |
| 24 | 16 |

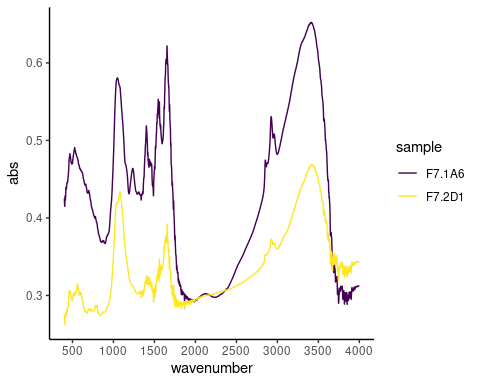
### Methods

Samples were ground with KBr and pressed under up to two tons of pressure. Repeated measurements were taken and after different grinding durations, and a grind curve was produced following Asscher et al. [-@asscherAtomicDisorder2011]. Samples were ground and analysed up to six times (a-f) for the grind curve. The analysis was conducted at Haifa University with a Thermo Nicolet is5 spectrometer in transmission, at 4 cm resolution, with an average of 32 scans between wavenumbers 4000 and 400 cm. The splitting factor was calculated following Asscher et al. [-@asscherAtomicDisorder2011], using the full width at half maximum (FWHM) of the main peak at 1035. Grinds a-f represent different levels of grinding. Samples denoted with an ‘a’ were ground for the least amount of time and ‘f’ the most.

### Results

# calculate wavenumber of highest peak  
# ftir\_data <- ftir\_data %>%  
# group\_by(sample) %>%   
# mutate(max\_peak = wavenumber[which(abs == max(abs))])  
grind\_sample\_order <- c(  
 "Archaeological calculus",  
 "Artificial calculus day 16",  
 "Artificial calculus day 20",  
 "Artificial calculus day 24",  
 "Archaeological bone",  
 "Bone-Dentine",  
 "Bone-Dentine\_2",  
 "Enamel",  
 "Enamel\_2",  
 "Enamel\_3")

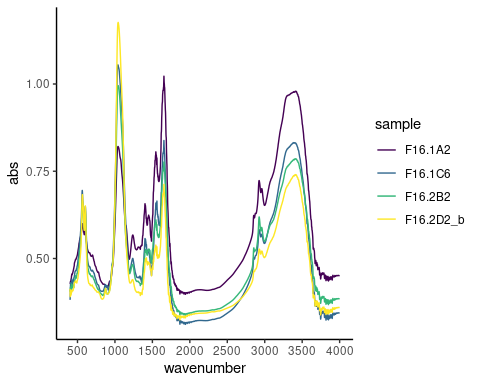
ftir\_data %>%  
 filter(  
 day == 7,  
 sample != "F7",  
 sample != "F7.1A6\_b"  
 ) %>%  
 ggplot(aes(x = wavenumber, y = abs, col = sample)) +  
 geom\_line() +  
 theme\_classic() +  
 scale\_x\_continuous(  
 n.breaks = length(as.character(seq(500, 4000, by = 500)))  
 )



Two spectra from biofilm sampled on day 7 of the experiment.

Day 7 spectra show large organic component, especially proteins (and collagen?). The hydroxyapatite peak is present at around 1040, but the doublet around 600 is poorly defined or absent.

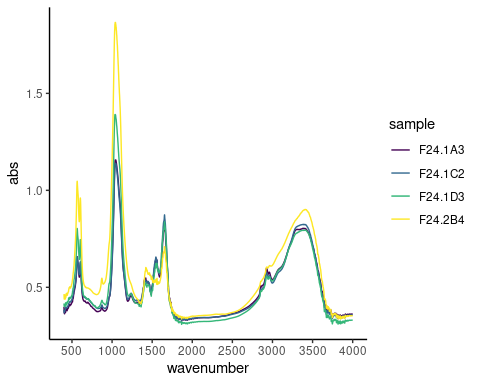
ftir\_data %>%  
 filter(  
 day == 16,  
 is.na(grind),  
 sample != "F16.2D2",  
 sample != "F16.1D6",  
 sample != "F16.1B2"  
 ) %>%  
 ggplot(aes(x = wavenumber, y = abs, col = sample)) +  
 geom\_line() +  
 theme\_classic() +  
 scale\_x\_continuous(  
 n.breaks = length(as.character(seq(500, 4000, by = 500)))  
 )



Spectra from biofilm sampled on day 16 of the experiment.

Day 16, the mineral phase has become more prominent than the organic. The hydroxyapatite peak is now the main peak, and the doublet is well-defined. There is still some variability in relative heights of the peaks between samples.

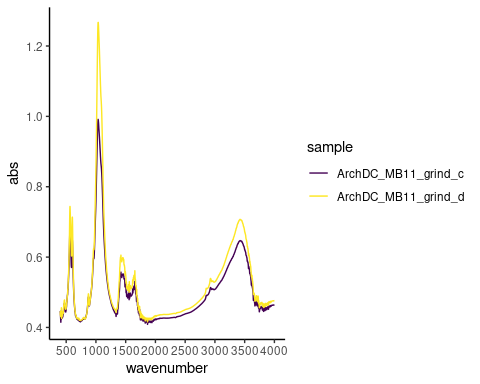
ftir\_data %>%  
 filter(  
 day == 24,  
 is.na(grind),  
 str\_detect(sample, "[\_]", negate = T),  
 sample != "F24.1B3",  
 sample != "F24.2C3",  
 sample != "F24.2D3",  
 sample != "F24.2A4",  
 ) %>%  
 ggplot(aes(x = wavenumber, y = abs, col = sample)) +  
 geom\_line() +  
 theme\_classic() +  
 scale\_x\_continuous(  
 n.breaks = length(as.character(seq(500, 4000, by = 500)))  
 )



Two spectra from the final biofilm product sampled on day 24 of the experiment.

Day 24, the mineral phase continues to dominate. The main peak is the hydroxyapatite peak at around 1039 with a well-defined doublet at 604 and 565.

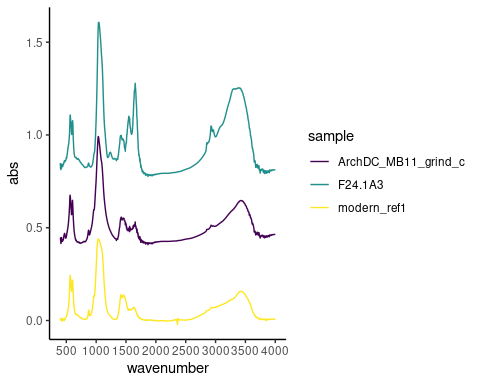
ftir\_data %>%  
 filter(  
 str\_detect(sample, "MB11\_grind\_[cd]")  
 ) %>%  
 ggplot(aes(x = wavenumber, y = abs, col = sample)) +  
 geom\_line() +  
 theme\_classic() +  
 scale\_x\_continuous(  
 n.breaks = length(as.character(seq(500, 4000, by = 500)))  
 )



Two spectra from a single sample of archaeological calculus with different grinding levels (c and d).

The reference calculus is similar to the later biofilm samples. The main difference is the a lower protein component seen as a lower Amide I peak at around 1637.

ftir\_data %>%  
 filter(  
 str\_detect(sample, "MB11\_grind\_c") | sample == "F24.1A3" |   
 sample == "modern\_ref1"  
 ) %>%  
 mutate(abs = case\_when(sample == "F24.1A3" ~ abs + 0.45,  
 TRUE ~ abs)) %>%   
 ggplot(aes(x = wavenumber, y = abs, col = sample)) +  
 geom\_line() +  
 theme\_classic() +  
 scale\_x\_continuous(  
 n.breaks = length(as.character(seq(500, 4000, by = 500)))  
 )



Spectra from archaeological calculus, modern reference calculus, and artificial calculus are compared. Absorbance was shifted to allow comparison of the three spectra, so the absorbance should be interpreted as relative, not absolute.

The carbonate peak at 1421 is not as well-developed as the reference spectrum. Protein at 1654.

The reference spectrum consists of carbonate (peaks 1418, 1458, 876), hydroxyapatite (peaks 1036, 604, 566), and protein (1637, Amide I).

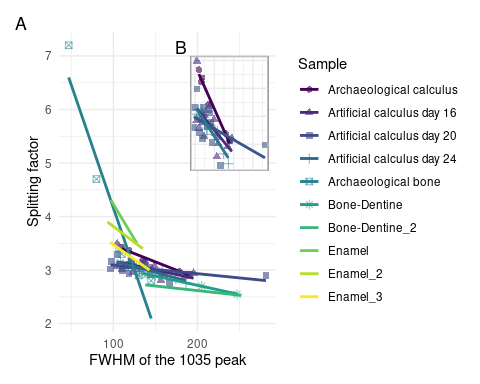
#### Grind curves

Splitting factors were calculated from the grinding measurements, and a grinding curve was created to explore crystallinity (crystal size) order, and disorder. Disorder is a steep slope and large FWHM.

Samples are similar to fresh bone and dentin. No appreciable differences between days 16, 20, and 24.

grind\_all\_plot <- grind\_data %>%  
 mutate(  
 Sample\_day = factor(Sample\_day, levels = grind\_sample\_order)) %>%  
 group\_by(day, Sample) %>%  
 ggplot(aes(x = FWHM, y = IRSF, col = Sample\_day, shape = Sample\_day)) +  
 geom\_point(size = 2, alpha = 0.6) +  
 geom\_smooth(method = "lm", se = F) +  
 theme\_minimal() +  
 labs(col = "Sample", shape = "Sample",  
 x = "FWHM of the 1035 peak",  
 y = "Splitting factor")  
  
# isolate calculus samples to see diffs between days and the 'real deal'  
grind\_calc\_plot <- grind\_data %>%  
 filter(Sample == "Artificial calculus" | Sample == "Archaeological calculus") %>%  
 ggplot(aes(x = FWHM, y = IRSF, col = Sample\_day, shape = Sample\_day)) +  
 geom\_point(size = 2, alpha = 0.6) +  
 geom\_smooth(method = "lm", se = F) +  
 labs(col = "Sample", shape = "Sample",  
 x = "FWHM of the 1035 peak",  
 y = "Splitting factor") +  
 theme\_minimal() +  
 theme(  
 axis.text = element\_blank(),  
 axis.title = element\_blank(),  
 axis.ticks = element\_blank(),  
 legend.position = "none",  
 panel.border = element\_rect(  
 colour = "grey",   
 fill = "transparent", size = 1),  
 panel.background = element\_rect(fill = "white")  
 ) +  
 scale\_colour\_viridis\_d(end = 0.4)

grind\_all\_plot + inset\_element(grind\_calc\_plot, left = 0.5, bottom = 0.5, right = 1, top = 1) +   
 plot\_layout(guides = "collect") + plot\_annotation(tag\_levels = "A")



(A) Grinding curves of multiple materials; and (B) calculus-only materials, including biofilm samples from three days, and an archaeological calclulus sample.

## Conclusion

The artificial samples from day 24 resemble both the modern reference samples and the archaeological sample in mineral composition and crystallinity. Overall, the spectra of the artificial calculus show an increase in the inorganic component and a decrease in the organic protein component over time. The grinding curves for days 16, 20, and 24 were very similar, suggesting that the CPMU solution (introduced on day 16) may not have much of an effect.