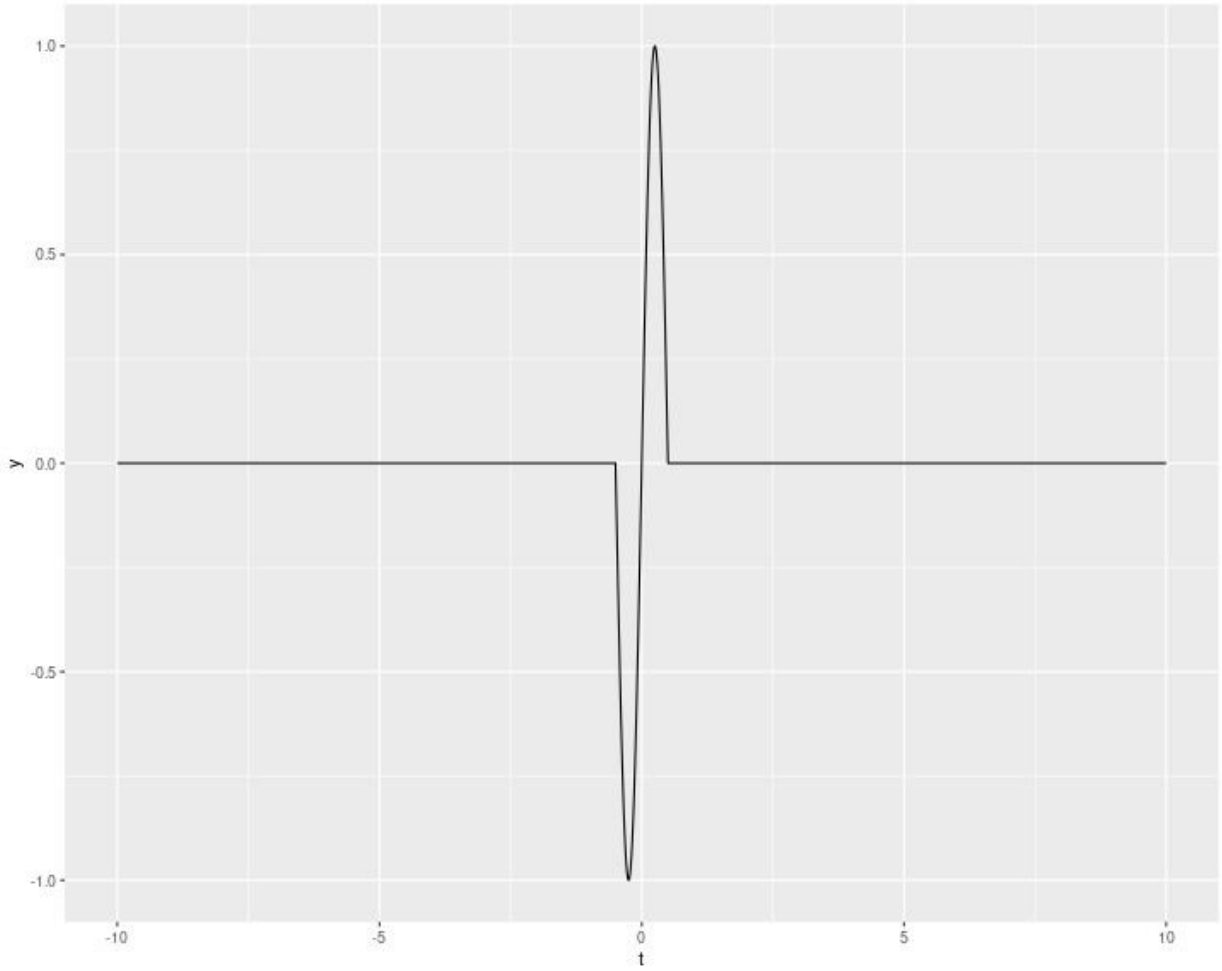
Some Basic Stuff and Sanity Checking

Let's make a function that is a sin function for $\ell \le x \le \ell$ and is otherwise 0.

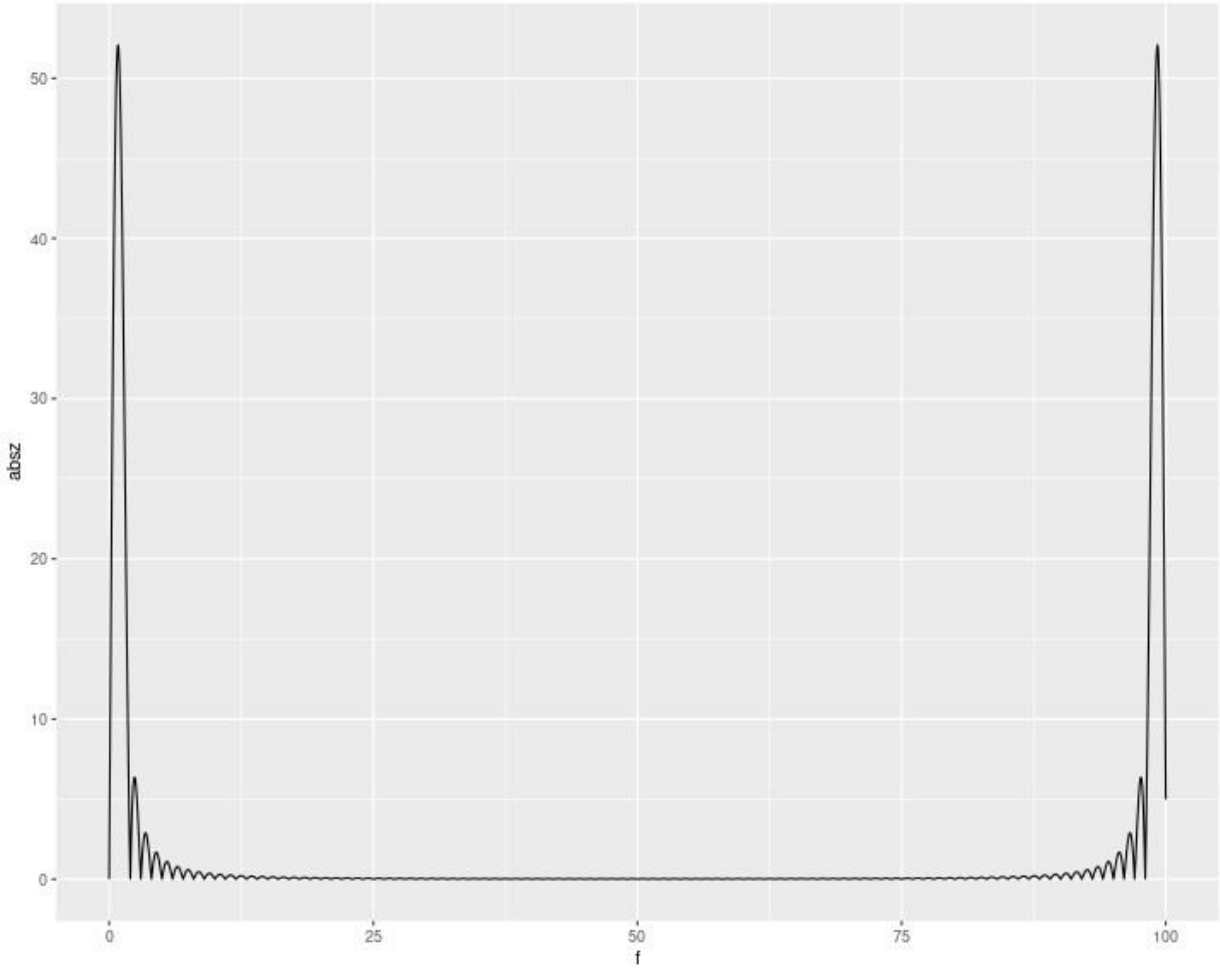
```
f1 <- function(t, l) {
  if (t < -l | t > l) {
    res <- 0
  } else {
    res <- sin(2*pi * t)
  }
  return(res)
}
f1v <- Vectorize(f1)
```

And now let's examine this function for $-10 < t < 10$ and $\ell = 1$



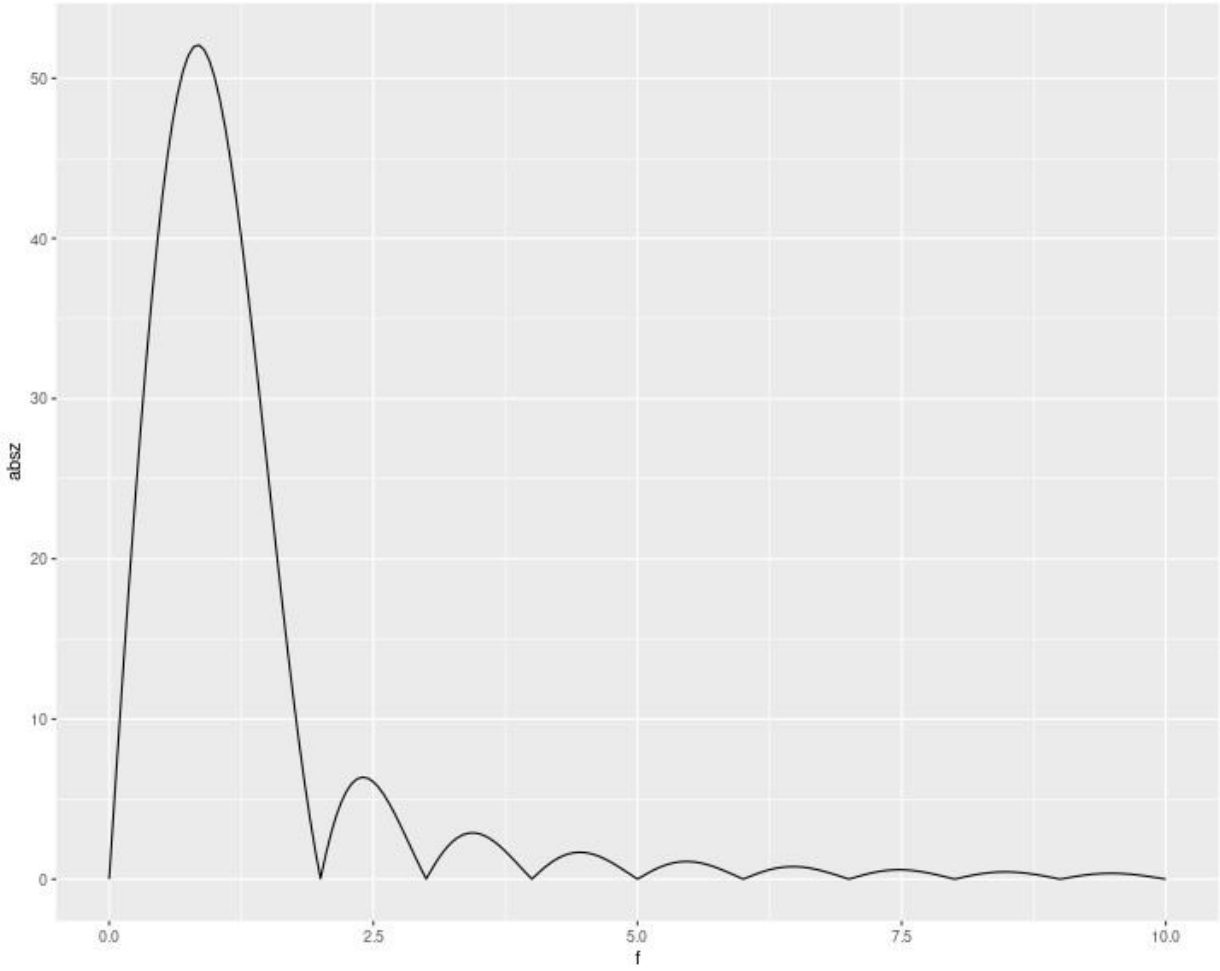
The FFT of this function can be determined with the R `fft()` function. The differential Δf is calculated with the formula

$$\Delta f = \frac{1}{N \Delta t} $$



Zooming in a bit we have:

```
df %>%
  ggplot(aes(x = f, y = absz)) +
  geom_line() +
  xlim(c(0,10))
```

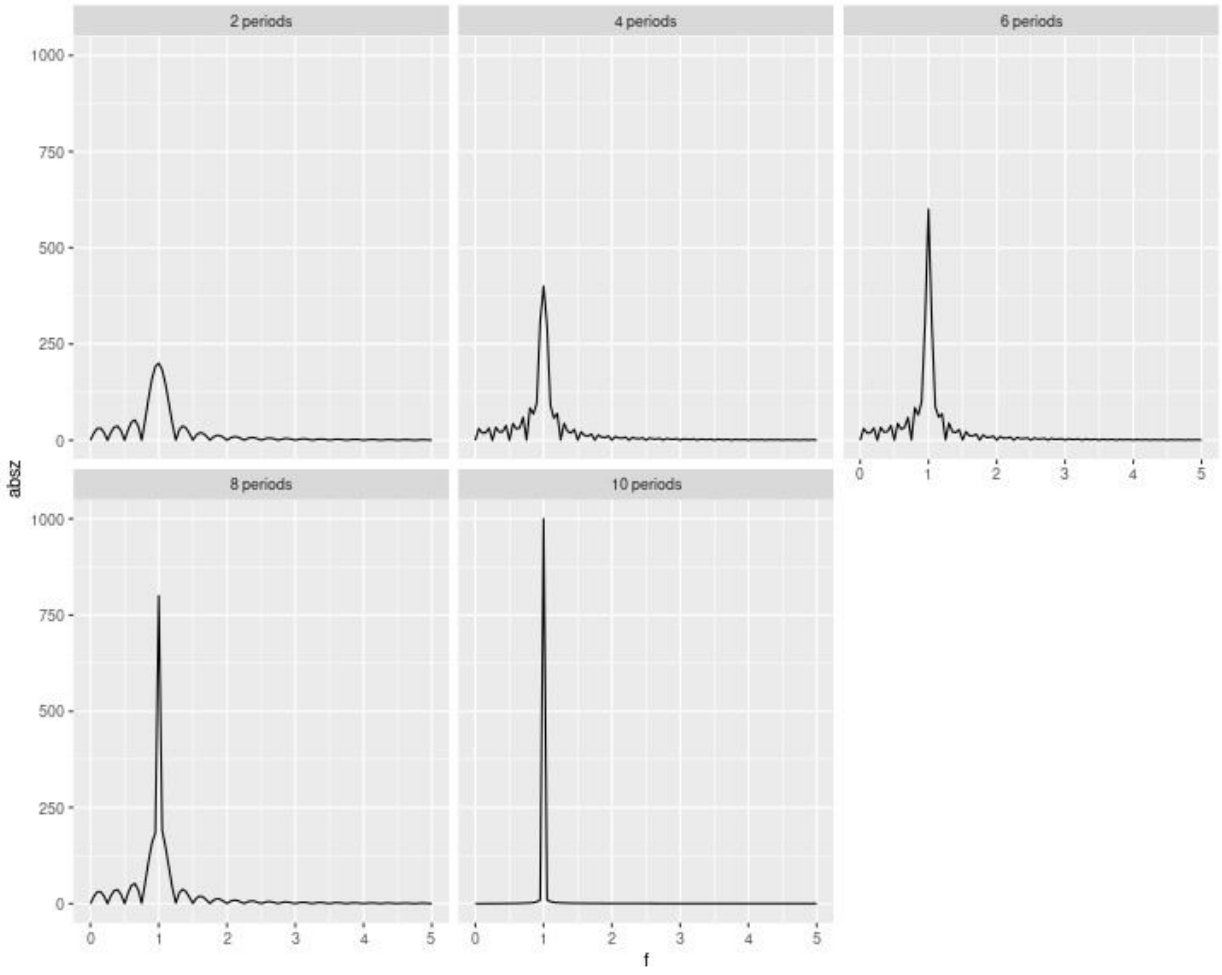


This looks as it should – trying to be a delta function, namely \(\delta(x – 1)\). As we increase the number of periods we feed the FFT the better this becomes:

```
df <- NULL
tmp <- data.frame(t = t, y = rep(NA,N), z = rep(NA,N), absz = rep(NA,N), f =
rep(NA,N), nT = rep(NA,N))

for(i in 1:5) {
  tmp <- tmp %>%
    mutate(y = f1v(t, 2 * i)) %>%
    mutate(z = fft(y)) %>%
    mutate(absz = abs(z)) %>%
    mutate(deltaf = 1 / (N * ((b - a) / N))) %>%
    mutate(f = (1:N - 1) * deltaf) %>%
    mutate(nT = factor(paste(2 * i, "periods"), levels = paste(2 * i, "periods")))
  df <- bind_rows(df, tmp)
}
```

So as the number of periods increase, things improve and by 10 periods, we have a very sharp \(\delta\) function.

```
df %>%
  group_by(nT) %>%
  ggplot(aes(x = f, y = absz)) +
  geom_line() +
  xlim(c(0,5)) +
  facet_wrap(~nT)
```

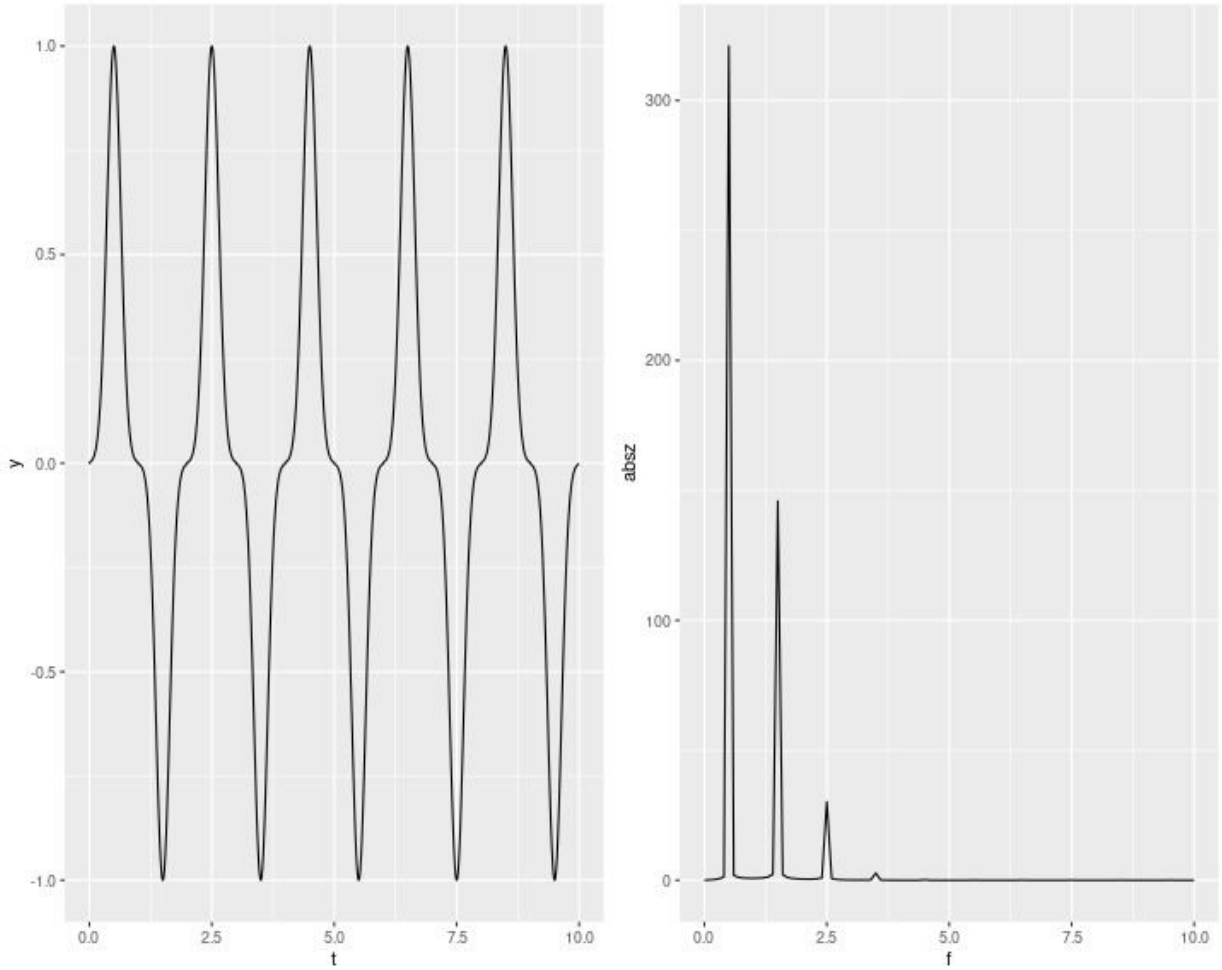


Applying FFT to a Gaussian

We can do the same thing to a Gaussian to mimic the gamma region of electrophoresis. To do this we just replicate the gamma function, inverting it in order to make the resulting function odd. Let's just examine 5 periods for simplicity which is 10 replications of the gamma region.

```
library(gridExtra)
N <- 1000
a <- 0
b <- 10
t <- seq(a, b, length.out = N)

f2 <- function(x) {
  remainder <- x - trunc(x)
  if (ceiling(x) %% 2 == 1) {
    res <- +exp(-25 * (remainder - 0.5) ^ 2)
  } else {
    res <- -exp(-25 * (remainder - 0.5) ^ 2)
  }
  return(res)
}

f2v <- Vectorize(f2)
df <- data.frame(t = t, y = rep(NA,N), z = rep(NA,N), absz = rep(NA,N), f =
rep(NA,N))
df <- df %>%
  mutate(y = f2v(t)) %>%
  mutate(z = fft(y)) %>%
  mutate(absz = abs(z)) %>%
  mutate(deltaf = 1/(N*((b-a)/N))) %>%
  mutate(f = (1:N - 1)*deltaf)
```

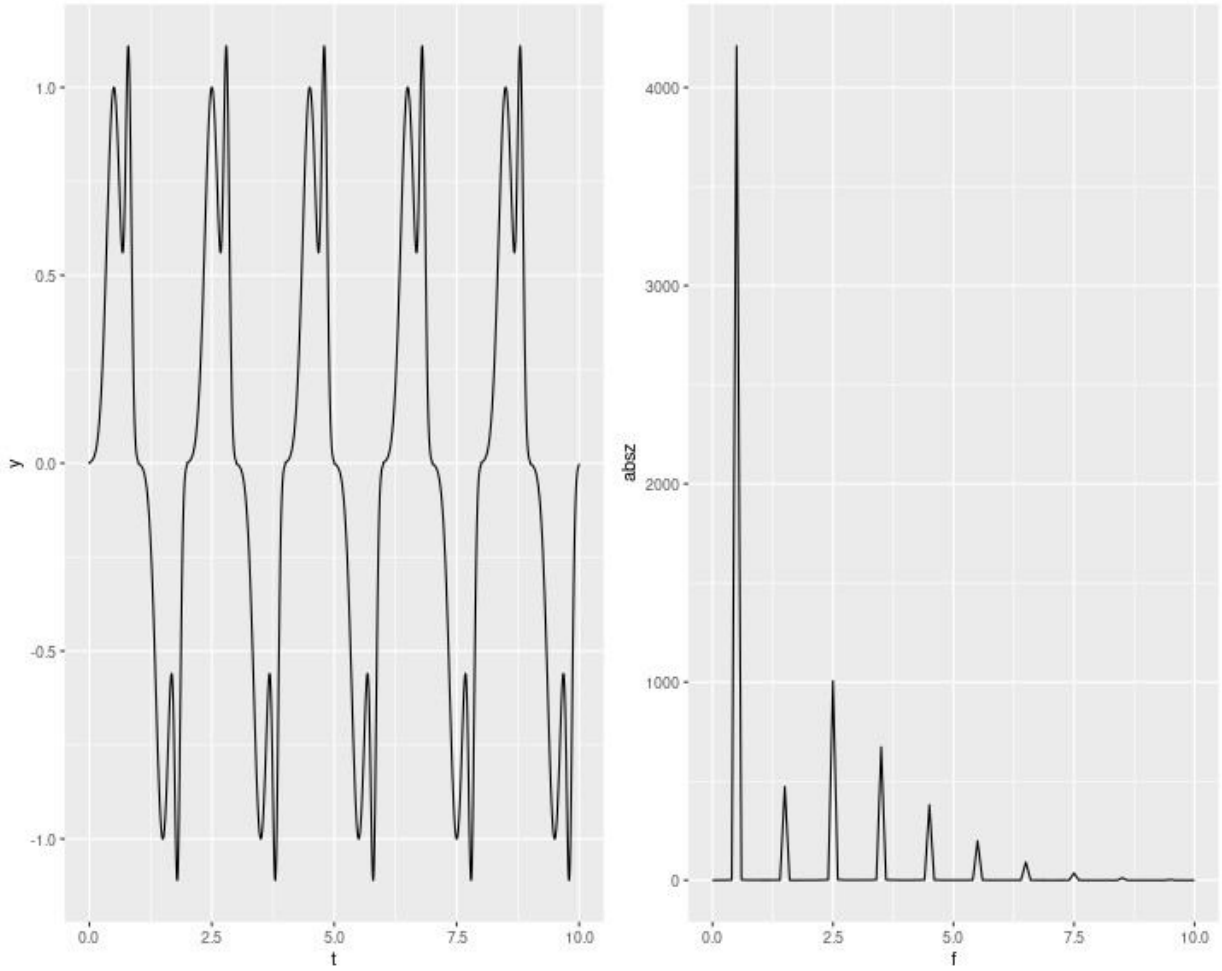
```
p1 <- df %>%
  ggplot(aes(x = t, y = y)) +
  geom_line() +
  xlim(c(0,b))

p2 <- df %>%
  ggplot(aes(x = f, y = absz)) +
  geom_line() +
  xlim(c(0,b))

grid.arrange(p1, p2, ncol=2)
```



So, with a pure Gaussian, we more or less have 4 peaks in the FFT. If we now mimic a monoclonal protein by adding a second Gaussian, we can see that the FFT becomes more complex because more frequencies (i.e. \(n\)) of \(e^{\frac{-2 \pi i}{N}kn}\) are required to characterize the concatenated periodic function.

```
N <- 10000
a <- 0
b <- 10
t <- seq(a, b, length.out = N)

f3 <- function(x){
  remainder <- x - trunc(x)
  if(ceiling(x) %% 2 == 1){
  res <- exp(-25*(remainder-0.5)^2) + exp(-150*(remainder-0.8)^2)
  } else {
  res <- -exp(-25*(remainder-0.5)^2) - exp(-150*(remainder-0.8)^2)
  }
  return(res)
}
```

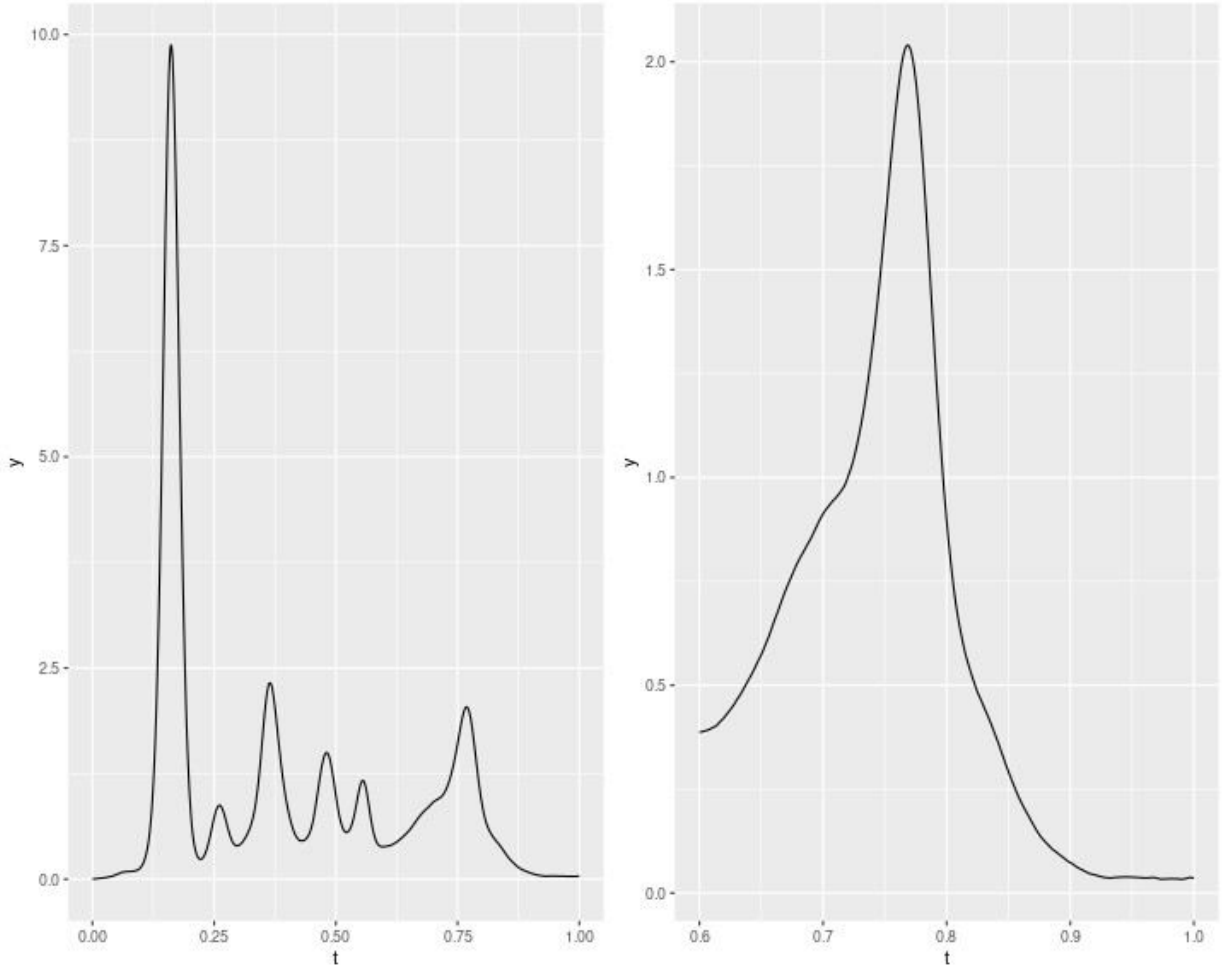
```
f3v <- Vectorize(f3)
df <- data.frame(t = t, y = rep(NA,N), z = rep(NA,N), absz = rep(NA,N), f =
rep(NA,N))
df <- df %>%
  mutate(y = f3v(t)) %>%
  mutate(z = fft(y)) %>%
  mutate(absz = abs(z)) %>%
  mutate(deltaf = 1/(N*((b-a)/N))) %>%
  mutate(f = (1:N - 1)*deltaf)

p1 <- df %>%
  ggplot(aes(x = t, y = y)) +
  geom_line() +
  xlim(c(0,b))

p2 <- df %>%
  ggplot(aes(x = f, y = absz)) +
  geom_line() +
  xlim(c(0,b))

grid.arrange(p1, p2, ncol=2)
```



The gamma region with a monoclonal results in an obviously more complex FFT.

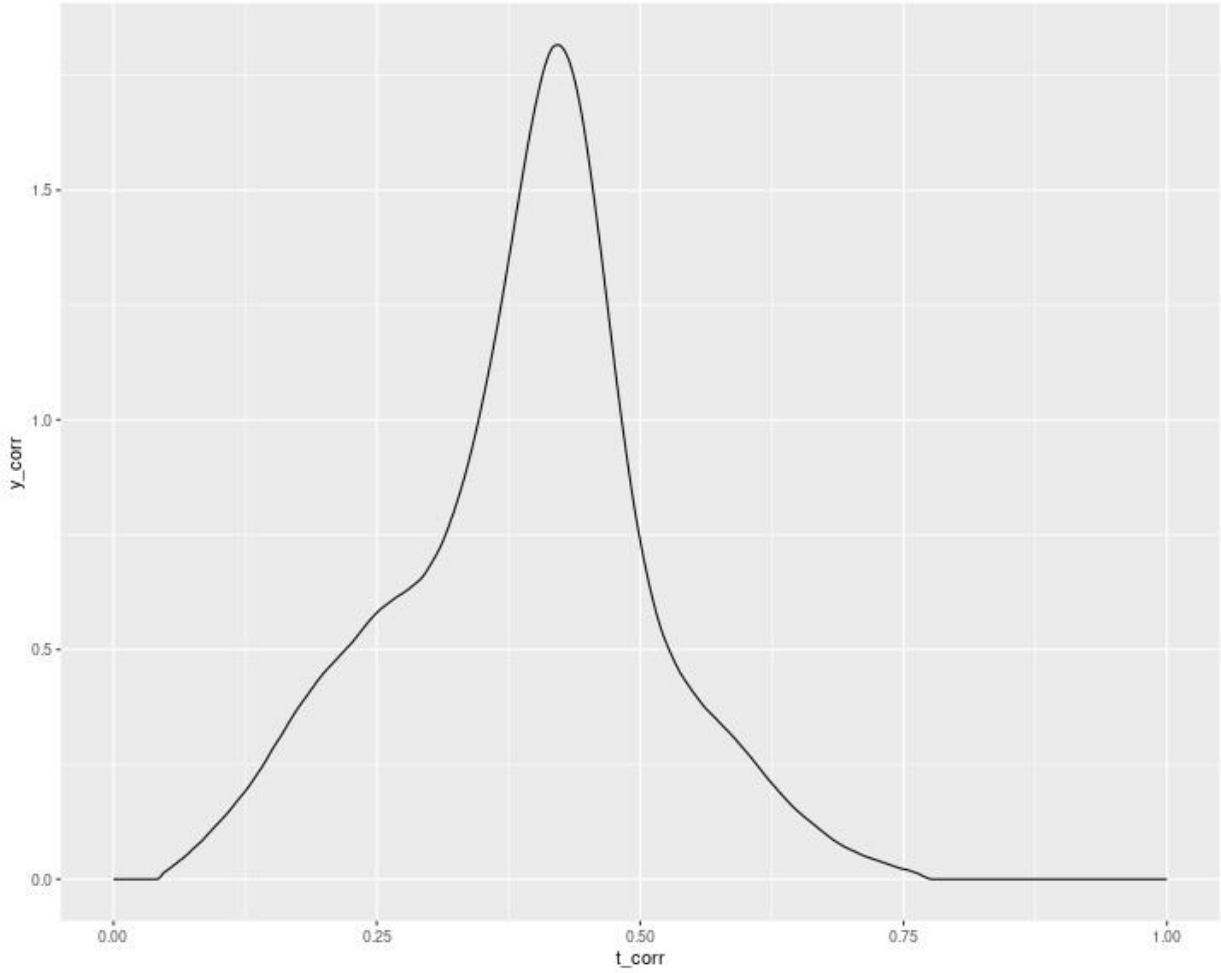
Apply to a Real Gamma Region

Here is an example densitometric scan. We can pull out the gamma region.



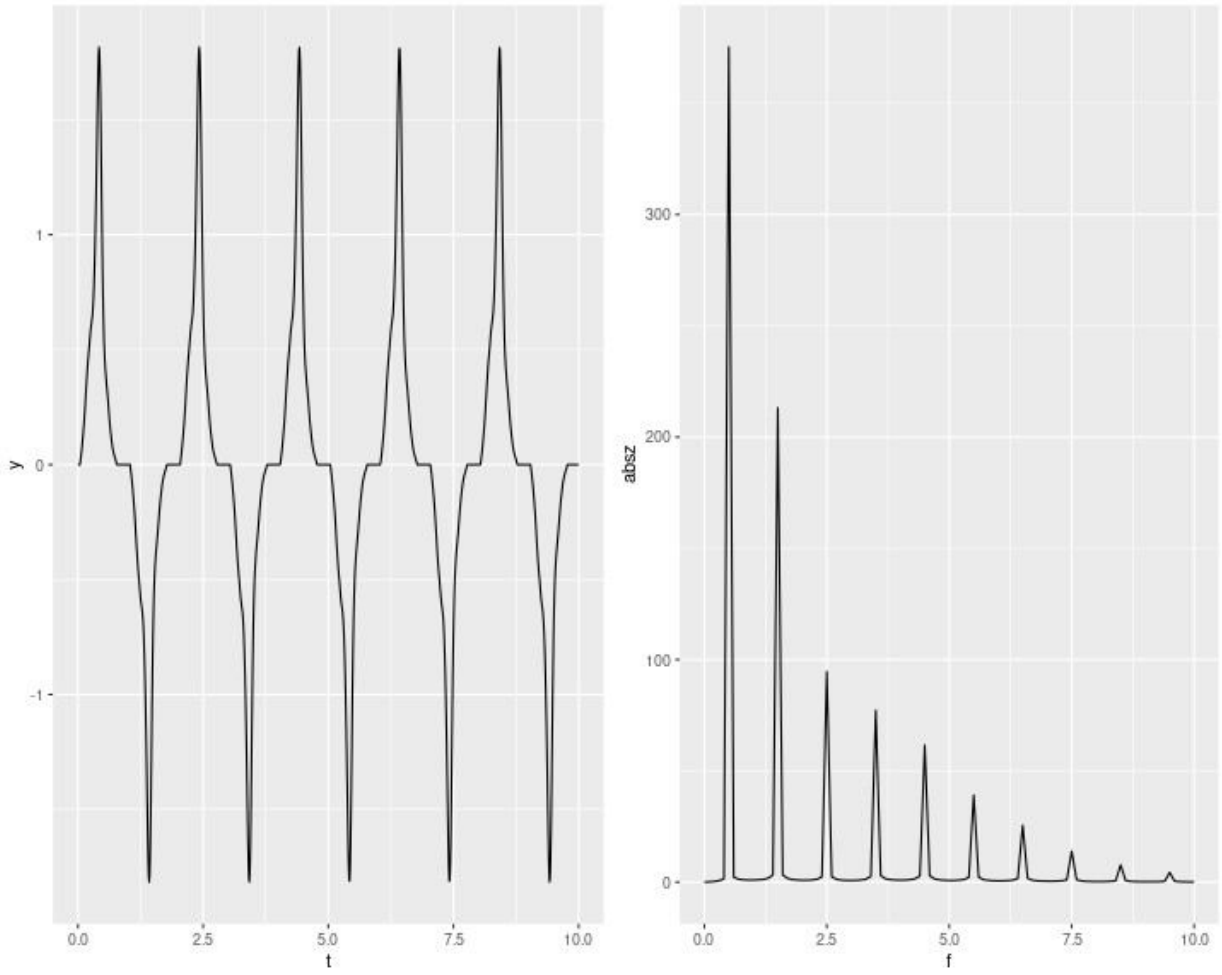
Then we can baseline the gamma region, replicate it 10 times and apply the FFT.

```
library(baseline)
N <- 1000
a <- 0
b <- 10
t <- seq(a, b, length.out = N)

gamma <- filter(real_data, t > 0.6)

bl <- baseline(matrix(gamma$y, nrow = 1), method = "modpolyfit", degree = 1)
gamma$y_corr <- bl@corrected[1,]
gamma$y_corr[gamma$y_corr < 0.01] <- 0
gamma$y_corr <- stats::filter(gamma$y_corr, rep(0.2,5), circular = TRUE) # force a
little smoothing to get rid of cusps

#normalize the domain
gamma$t_corr <- (gamma$t - head(gamma$t,1))/(tail(gamma$t,1) - head(gamma$t,1))

gamma %>%
  ggplot(aes(x = t_corr, y = y_corr)) +
  geom_line()
```




```
# make a spline function
ep_fun <- splinefun(gamma$t_corr, gamma$y_corr)

f4 <- function(x){
  remainder <- x - trunc(x)
  if(ceiling(x) %% 2 == 1){
  res <- ep_fun(remainder)
  } else {
  res <- -ep_fun(remainder)
  }
  return(res)
}

f4v <- Vectorize(f4)

df <- data.frame(t = t, y = rep(NA,N), z = rep(NA,N), absz = rep(NA,N), f =
rep(NA,N))
df <- df %>%
  mutate(y = f4v(t)) %>%
  mutate(z = fft(y)) %>%
  mutate(absz = abs(z)) %>%
  mutate(deltaf = 1/(N*((b-a)/N))) %>%
  mutate(f = (1:N - 1)*deltaf)

p1 <- df %>%
  ggplot(aes(x = t, y = y)) +
  geom_line() +
  xlim(c(0,b))

p2 <- df %>%
```

```
  ggplot(aes(x = f, y = absz)) +
  geom_line() +
  xlim(c(0,b))

grid.arrange(p1, p2, ncol=2)
```



And that is more or less the gist of things. The simpler the gamma region is, the fewer peaks there will be in the frequency domain. This permits recapitulation of what Kratzer et al did in their 1992 paper with modern techniques. As I say, I am not sure its necessary but it might improve model performance.…