

Statistics_Ch3_Proteorhodopsin

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Statistics: Plates (Blanks)

DFOB - 0, 10, 50, 100 umol Light - blue and dark

```
rm(list=ls())
setwd("~/Desktop/MANUSCRIPT /CH4 Proteorhodopsin/Rscripts/Figure 3.1_stat")
data=read.table('white_plate.txt', na.strings="NA", sep='\t', header=T, dec=',')
head(data) # inspect data
```

```
##      Iron Light Well X1 X2 X3 X4 X5 X6 X7 X8 X9 X10 X11 X12 X13 X14 X15 X16
## 1    50  dark    A1 29 29 29 29 35 17 35 29 29 41 23 23 23 41 17 70
## 2   100  dark    A3 29 41 52 23 41 29 47 41 58 23 29 23 23 58 35 41
## 3    50  dark    A4 35 47 70 52 47 58 23 41 47 35 35 35 47 35 47 87
## 4   100  dark    A6 23 23 23 35 29 47 29 23 29 29 23 52 29 35 17 35
## 5    50  dark    A7 23 64 35 35 47 35 35 35 23 29 41 29 23 35 23 23
## 6   100  dark    A9 35 23 29 47 23 58 12 23 35 17 29 23 52 23 41 29
##      X17 X18 X19 X20 X21 X22 X23 X24 X25 X26 X27 X28 X29 X30 X31 X32 X33 X34
## 1   35  47  35  29  29  29  17  35  41  17  29  29  23  23  29  35  41  23
## 2   35  29  35  35  23  41  41  47  35  41  41  23  29  29  29  35  29  41
## 3   35  47  47  35  29  35  47  29  35  47  29  23  41  17  29  35  23  41
## 4   23  29  23  29  35  35  35  47  17  29  17  23  41  47  23  17  47  41
## 5   29  29  29  47  23  29  35  41  35  29  35  29  29  23  29  17  29  29
## 6   41  23  35  41  29  35  58  41  41  23  99  41  35  41  23  29  23  41
##      X35 X36 X37 X38 X39 X40 X41 X42 X43 X44 X45 X46 X47 X48      average
## 1   29  23  23  29  29  29  47  35  35  29  41  23  29  29 30.72916667
## 2   29  41  41  76  29  29  17  29  35  23  35  23  23  29 34.79166667
## 3   17  41  35  29  41  23  58  29  35  23  23  29  29  47      38
## 4   29  41  23  29  23  29  35  23  41  23  29  23  35  47 30.60416667
## 5   52  47  23  47  29  29  35  35  29  29  29  29  23  41 32.45833333
## 6   23  35  29  29  29  41  29  23  23  29  35  17  23  17 32.91666667
```

2-way Anova White Plate

```
data1 = gather(data, key=Time, value=Luminescence, 4:51 )
head(data1)
```

```
##      Iron Light Well      average Time Luminescence
## 1    50  dark    A1 30.72916667    X1              29
## 2   100  dark    A3 34.79166667    X1              29
## 3    50  dark    A4      38      X1              35
## 4   100  dark    A6 30.60416667    X1              23
## 5    50  dark    A7 32.45833333    X1              23
## 6   100  dark    A9 32.91666667    X1              35
```

```
data1<-na.omit(data1) #remove any present NA values
```

```

data1$loglum <-log(data1$Luminescence)

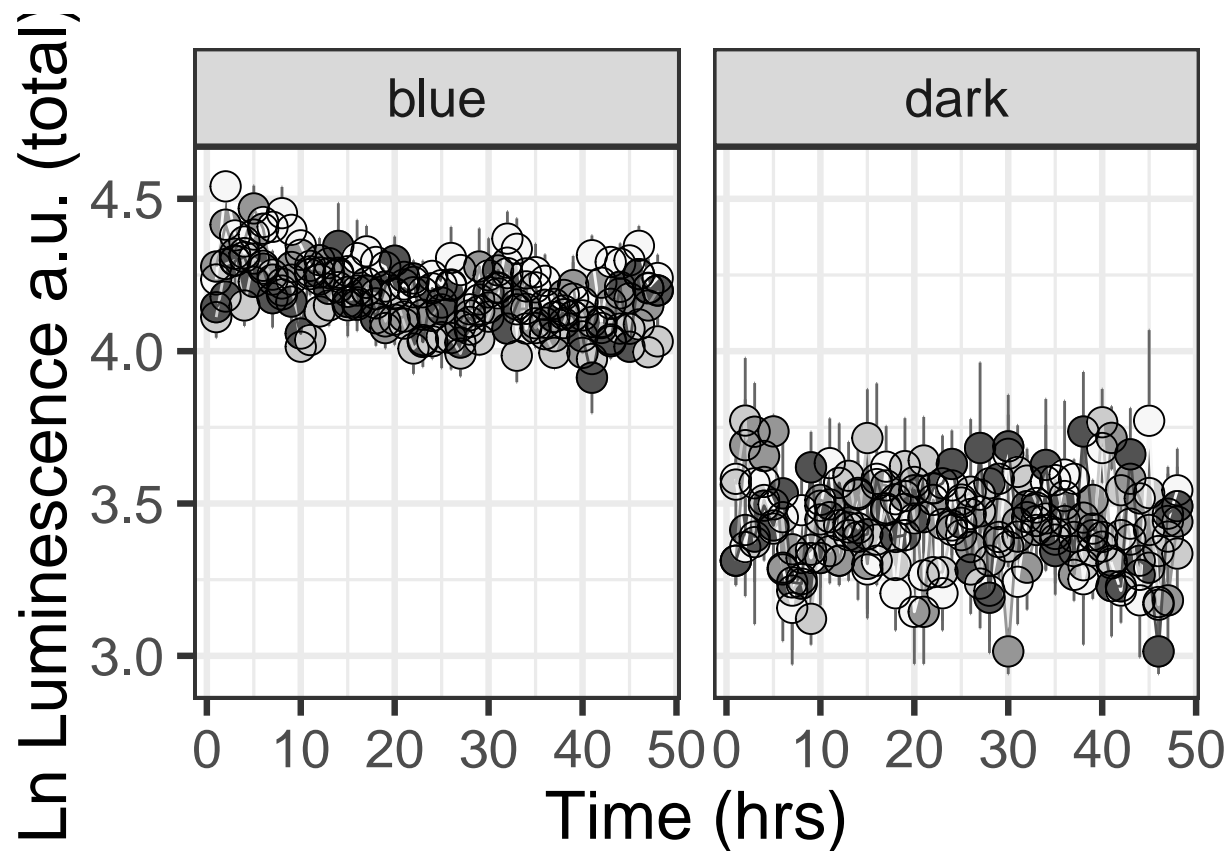
mean_name= ddpoly(data1,.(Light, Time, Iron), #all different groupings required
               summarize, meanvalue=mean(loglum),
               SE = sd(loglum) / sqrt((length(loglum))))
head(mean_name)

##   Light Time Iron meanvalue      SE
## 1  blue  X1    0  4.235360 0.07235102
## 2  blue  X1   10  4.111672 0.06750724
## 3  blue  X1   50  4.277686 0.02919078
## 4  blue  X1  100  4.144249 0.08652694
## 5  blue X10    0  4.347324 0.06853523
## 6  blue X10   10  4.014611 0.04180983

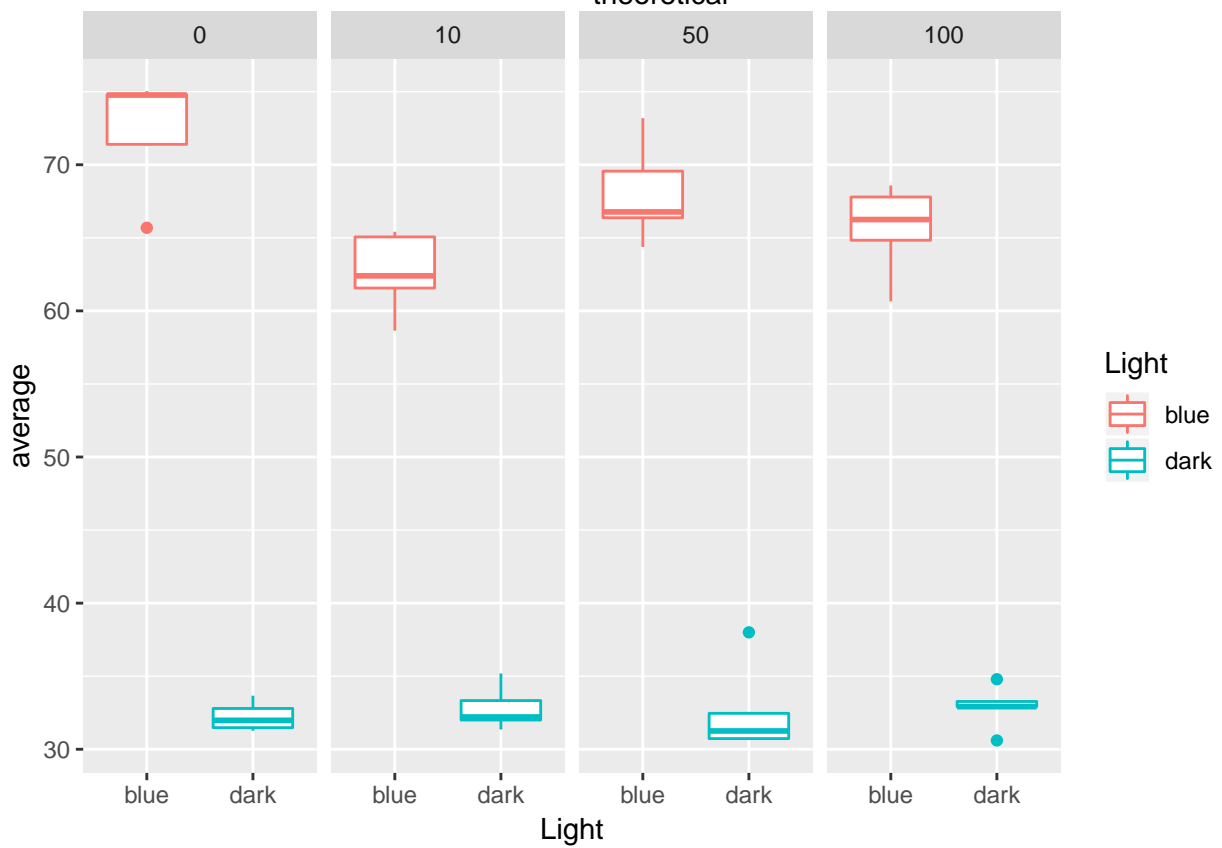
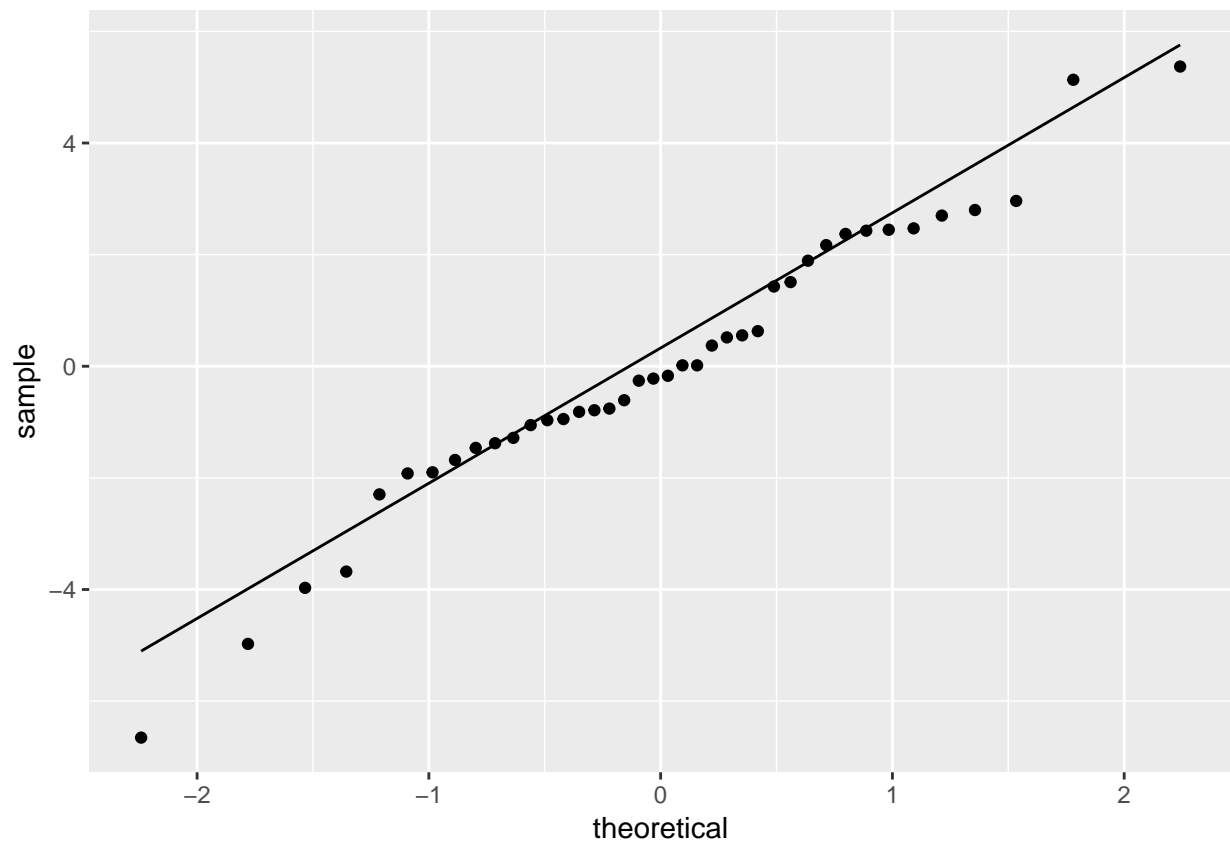
mean_name$Time <- gsub("X","",mean_name$Time) #removes X from the time column
mean_name$Time <- as.numeric(as.character(mean_name$Time))
mean_name$meanvalue <- as.numeric(as.character(mean_name$meanvalue))
mean_name$Light <- as.factor(mean_name$Light)
mean_name$Iron <- as.factor(mean_name$Iron)

curve = ggplot(data=mean_name, aes(x=Time, y= meanvalue, colour=Iron))
curve = curve + geom_errorbar(aes(ymin=meanvalue-SE, ymax=meanvalue+SE),
                               colour="grey40",
                               width = 0.08)
curve = curve + geom_point(size=5)+geom_line()
curve = curve + theme_bw(base_size= 25) + ylab("Ln Luminescence a.u. (total)") + xlab ("Time (hrs)")
curve=curve+facet_grid(.~Light)
#curve=curve+guides(colour=guide_legend(title=bquote('DFOB ('*mu~'mol)')))
#curve = curve + scale_y_continuous(limits=c(0, 3000))
curve = curve + scale_colour_manual(values = c("#f7f7f7", "#cccccc", "#969696", "#525252"), guide=FALSE) #
curve = curve + geom_point(shape = 1,size = 5,colour = "black")
curve

```



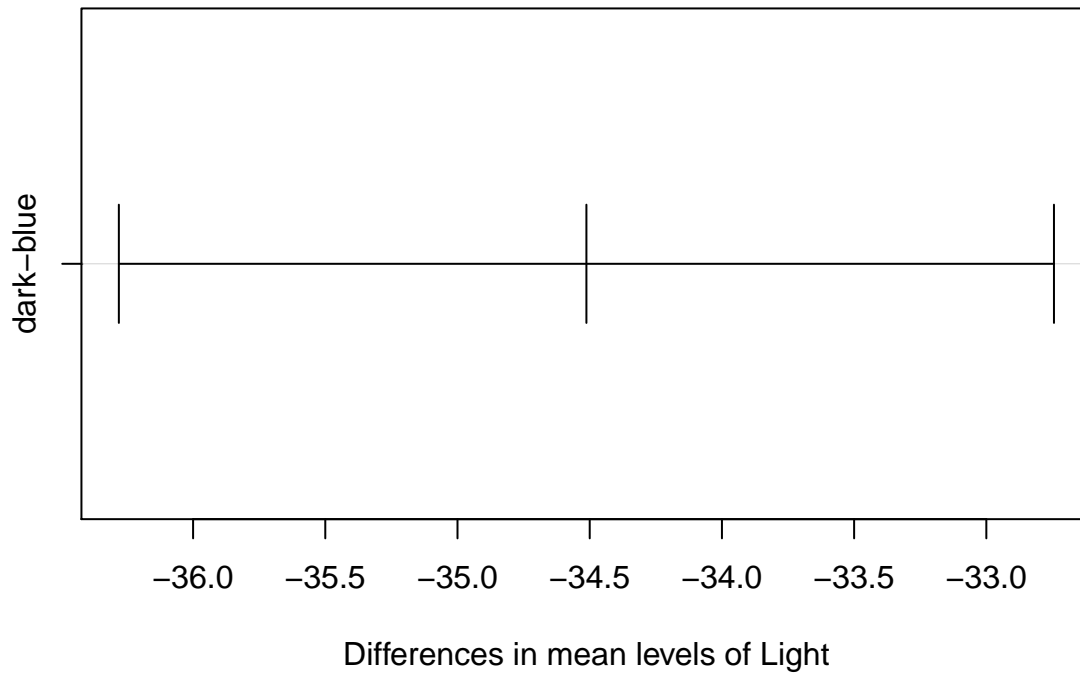
```
##           Df Sum Sq Mean Sq  F value    Pr(>F)
## Iron           3      111       37    4.901 0.00648 **
## Light          1    11911   11911 1580.536 < 2e-16 ***
## Iron:Light      3      144       48    6.360 0.00166 **
## Residuals     32      241        8
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



Tukey multiple comparisons of means

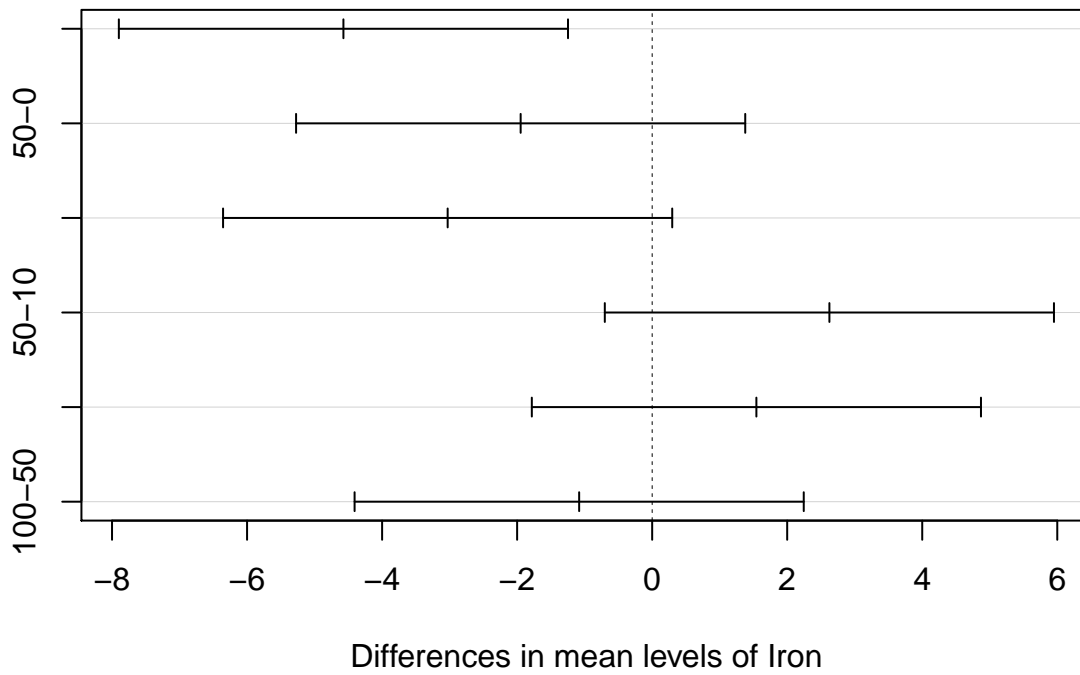
```
##      95% family-wise confidence level
##
## Fit: aov(formula = average ~ Iron * Light, data = data)
##
## $Iron
##           diff           lwr           upr      p adj
## 10-0    -4.572917 -7.8991771 -1.2466562 0.0039959
## 50-0    -1.947917 -5.2741771  1.3783438 0.4002845
## 100-0   -3.029167 -6.3554271  0.2970938 0.0847026
## 50-10    2.625000 -0.7012604  5.9512604 0.1629125
## 100-10   1.543750 -1.7825104  4.8700104 0.5958962
## 100-50  -1.081250 -4.4075104  2.2450104 0.8147838
##
## $Light
##           diff           lwr           upr      p adj
## dark-blue -34.5125 -36.28078 -32.74422      0
##
## $`Iron:Light`
##           diff           lwr           upr      p adj
## 10:blue-0:blue    -9.72500000 -15.3491284  -4.100872 0.0000860
## 50:blue-0:blue    -4.28750000  -9.9116284   1.336628 0.2444827
## 100:blue-0:blue   -6.72083333 -12.3449617  -1.096705 0.0104764
## 0:dark-0:blue    -40.10416666 -45.7282950 -34.480038 0.0000000
## 10:dark-0:blue   -39.52500000 -45.1491284 -33.900872 0.0000000
## 50:dark-0:blue   -39.71250000 -45.3366284 -34.088372 0.0000000
## 100:dark-0:blue  -39.44166666 -45.0657950 -33.817538 0.0000000
## 50:blue-10:blue    5.43750000  -0.1866284  11.061628 0.0639660
## 100:blue-10:blue   3.00416667  -2.6199617   8.628295 0.6684370
## 0:dark-10:blue   -30.37916666 -36.0032950 -24.755038 0.0000000
## 10:dark-10:blue  -29.80000000 -35.4241284 -24.175872 0.0000000
## 50:dark-10:blue  -29.98750000 -35.6116284 -24.363372 0.0000000
## 100:dark-10:blue -29.71666666 -35.3407950 -24.092538 0.0000000
## 100:blue-50:blue  -2.43333333  -8.0574617   3.190795 0.8498903
## 0:dark-50:blue   -35.81666666 -41.4407950 -30.192538 0.0000000
## 10:dark-50:blue  -35.23750000 -40.8616284 -29.613372 0.0000000
## 50:dark-50:blue  -35.42500000 -41.0491284 -29.800872 0.0000000
## 100:dark-50:blue -35.15416666 -40.7782950 -29.530038 0.0000000
## 0:dark-100:blue  -33.38333333 -39.0074617 -27.759205 0.0000000
## 10:dark-100:blue -32.80416667 -38.4282950 -27.180038 0.0000000
## 50:dark-100:blue -32.99166667 -38.6157950 -27.367538 0.0000000
## 100:dark-100:blue -32.72083333 -38.3449617 -27.096705 0.0000000
## 10:dark-0:dark    0.57916666  -5.0449617   6.203295 0.9999723
## 50:dark-0:dark    0.39166666  -5.2324617   6.015795 0.9999981
## 100:dark-0:dark   0.66250000  -4.9616284   6.286628 0.9999310
## 50:dark-10:dark  -0.18750000  -5.8116284   5.436628 1.0000000
## 100:dark-10:dark  0.08333334  -5.5407950   5.707462 1.0000000
## 100:dark-50:dark  0.27083334  -5.3532950   5.894962 0.9999999
```

95% family-wise confidence level



NULL

95% family-wise confidence level



NULL

```
##
## Shapiro-Wilk normality test
##
## data: residuals(white96)
## W = 0.97368, p-value = 0.4667

## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 7  0.5169  0.815
##      32
```

Normality and Homogeneity of variance is met. Plot shows clear differences between blue and dark conditions and slight differences based on DFOB. Plotting the Tukey Test further reveals that Differences in mean levels of iron are concerning minor differences and can be ignored while large differences are visible between light and dark conditions.

2-way Anova White Plate

```
rm(list=ls())
setwd("~/Desktop/MANUSCRIPT /CH4 Proteorhodopsin/Rscripts/Figure 3.2_stat")
data=read.table('Dark_Plates.txt', na.strings="NA", sep='\t', header=T, dec=',')
head(data) # inspect data
```

```
##      Bact    Light Well X1 X2 X3 X4 X5 X6 X7 X8 X9 X10 X11 X12 X13 X14 X15
## 1 Blanks  0 blue   A2 29 29 35 12 23 35 41 23 29  23  17  23  23  17  12
## 2 Blanks  0 blue   A5 12 17 23 17 23 47 29 23 29  35  23  12  29  29  35
## 3 Blanks  0 blue   A8 23 41 17 35 23 41 12 29 17  23  17  17  23  29  23
## 4 Blanks  0 blue  A11 29 29 17 17 17 23 29 23 23  12  17  64  17  29  23
## 5 Blanks  0 blue  A14 35 17 23 29 23 35 35 35 29  23  35  17  35  29  17
## 6 Blanks 25 Blue   B2 35 17 23 41 17 17 52 41 29  41  23  17  29  23  47
##      X16 X17 X18 X19 X20 X21 X22 X23 X24 X25 X26 X27 X28 X29 X30 X31 X32 X33
## 1  35  35  23  47  23  17  23  23  12  23  17  29  29  12  23  17  17  35
## 2  35  23  35  12  23  17  35  23  12  12  23  29  41  29  23  29  35  17
## 3  23  23  35  35  29  17  35  29  12  29  23  23  23  17  29  47  23  29
## 4  23  17  23  12  29  23  29  23  17  23  23  29  23  23  35  35  35  29
## 5  17  29  29  17  23  17  17  23  35  17  29  12  29  35  12  29  29  23
## 6  23  17  23  17  17  29  17  17  29  23  17  29  17  35  17  23  35  23
##      X34 X35 X36 X37 X38 X39 X40 X41 X42 X43 X44 X45 X46 X47 X48 X49 X50 X51
## 1  35  23  35  23  17  47  17  23  17  12  17  17  17  29  29  29  29  12
## 2  23  12  35  23  17  23  41  47  29  29  29  29  35  17  17  35  23  23
## 3  17  12  41  41  17  23  17  23  17  17  47  23  17  29  23  47  29  23
## 4  23  23  17  29  35  35  52  41  23  17  29  29  47  17  23  23  17  29
## 5  29  23  12  29  29  23  29  17  17  17  41  29  23  35  35  17  23  23
## 6  35  17  23  23  29  47  17  70  17  17  29  12  29  29  29  41  29  17
##      X52 X53 X54 X55 X56 X57 X58 X59 X60 X61 X62 X63 X64 X65 X66 X67 X68 X69
## 1  35  41  23  12  17  17  23  29  12  17  17  29  35  23  29  47  23  93
## 2  47  23  35  23  29  35  12  6  29  47  12  29  23  17  12  35  41  17
## 3  29  23  64  17  29  12  17  29  17  23  23  23  23  23  17  23  12  23
## 4  17  47  23  35  23  12  23  23  17  12  23  35  35  35  41  41  17  17
## 5  29  23  23  29  12  17  35  17  29  35  35  29  23  23  23  23  29  17
## 6  17  23  47  35  23  23  6  23  23  17  23  29  29  29  23  29  17  23
##      X70 X71 X72 X73 X74 X75 X76 X77 X78 X79 X80 X81 X82 X83 X84 X85 X86 X87
## 1  17  41  23  17  23  17  23  17  52  35  35  29  17  29  41  23  12  23
## 2  23  35  41  17  23  17  29  29  17  17  12  23  12  17  41  29  17  23
```

```
## 3 35 29 17 35 35 41 41 35 23 47 12 17 29 29 17 29 23 23
## 4 23 23 29 29 12 47 23 41 35 17 23 17 23 17 23 17 29 17
## 5 29 23 17 17 23 29 23 17 29 52 12 17 17 23 29 29 12 23
## 6 17 23 17 35 52 35 23 17 23 23 17 23 12 29 29 17 12 17
## X88 X89 X90 X91 X92 X93 X94 X95 average
## 1 23 17 41 29 29 23 23 23 25.71578947
## 2 17 17 17 29 35 12 23 47 25.41052632
## 3 23 23 64 12 17 17 17 23 25.83157895
## 4 29 23 41 23 12 23 35 17 25.82105263
## 5 29 12 23 29 29 35 29 17 24.89473684
## 6 23 23 29 29 29 52 23 23 26.03157895
```

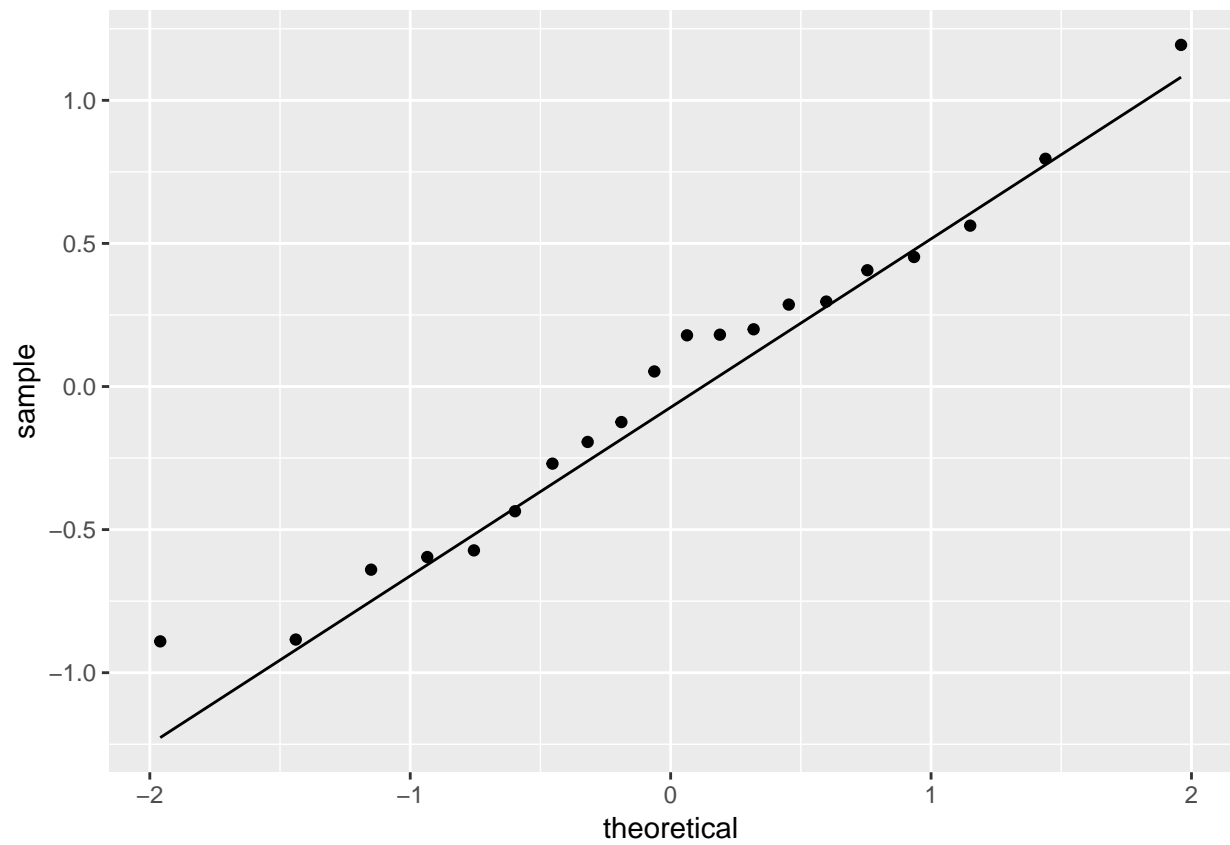
```
data$average <-as.numeric(as.character(data$average))
data$Light <-as.factor(data$Light)
```

```
dark96<-aov(average~Light,data=data)
summary(dark96) # sign treatment effect
```

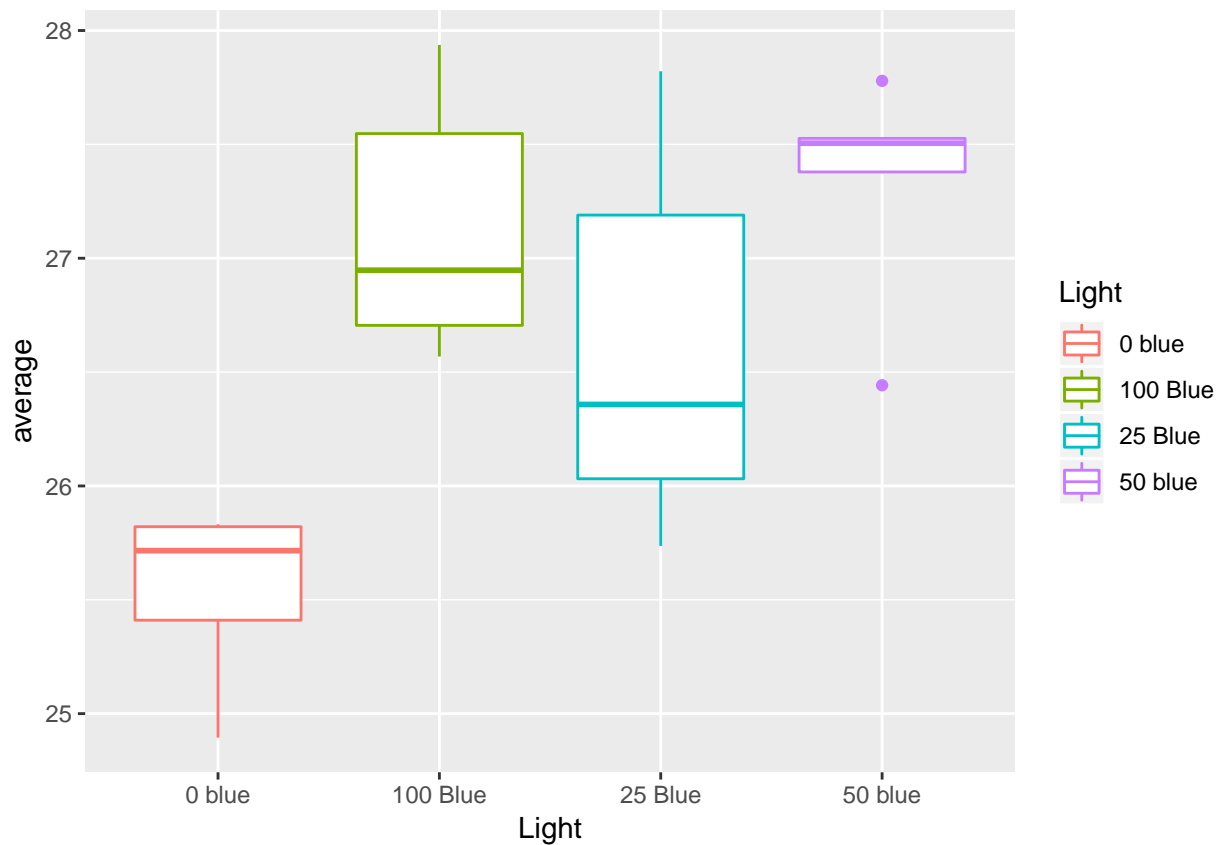
```
##           Df Sum Sq Mean Sq F value Pr(>F)
## Light      3  9.713   3.238   8.627 0.00123 **
## Residuals 16  6.005   0.375
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#Light: 0.00123**
```

```
delivery.res = data
delivery.res$M1.Fit = fitted(dark96)
delivery.res$M1.Resid = resid(dark96)
ggplot(delivery.res, aes(sample = M1.Resid)) + stat_qq() + stat_qq_line()
```

```
ggplot(data=data, aes(x=Light, y=average, colour=Light)) +geom_boxplot()
```



```
### normality ###
shapiro.test(residuals(dark96)) #ok

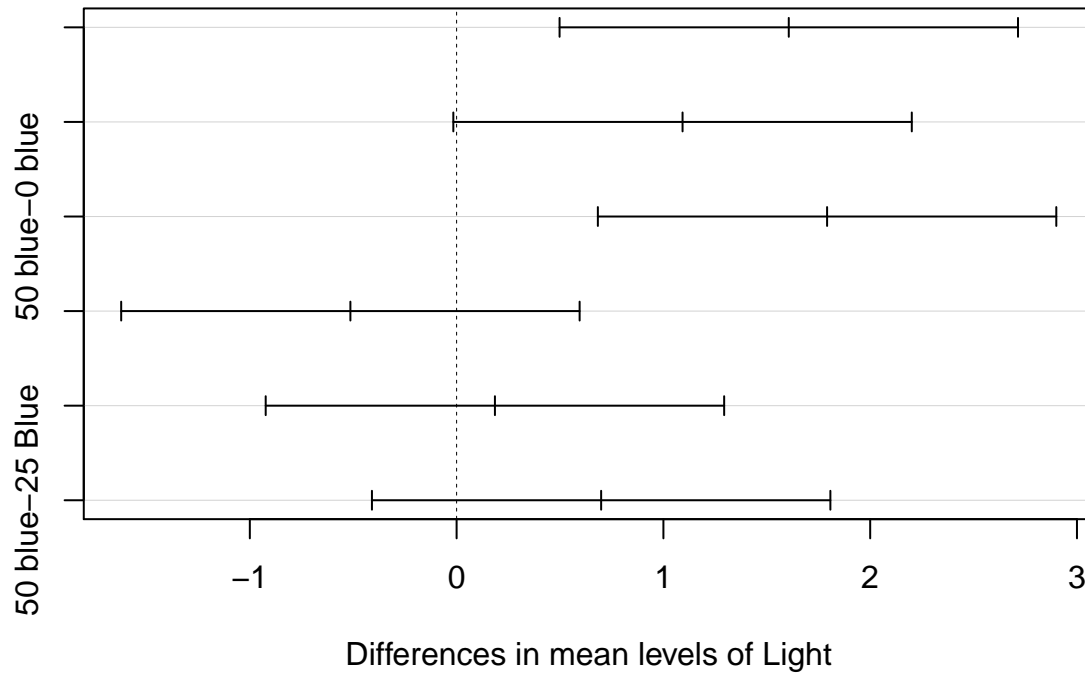
##
## Shapiro-Wilk normality test
##
## data: residuals(dark96)
## W = 0.97127, p-value = 0.7814

### homogeneity of variances ###
leveneTest(dark96) #ok

## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 3  0.8042 0.5097
##      16

##Plot TukeyHSD
p=plot(TukeyHSD(dark96, "Light"))
```

95% family-wise confidence level



p

NULL

Normality and Homogeneity of variance is met. Plot shows differences between dark and range of blue light conditions but Tuckey Test confirms that these mean differences are minor and can be ignored.

Statistics: Luminescence per Cell (Fe-replete and Fe-limiting conditions)

```
rm(list=ls())
setwd("~/Desktop/MANUSCRIPT /CH4 Proteorhodopsin/Rscripts/Figure 3.5_stat")
data=read.table('test_stats.txt', na.strings="NA", sep='\t', header=T, dec=',')
head(data) # inspect data
```

```
##      Time      Cond Light Iron      mL lum.cell lum.cell2
## 1      4      Fe Dark  Dark  Fe 2552750.00    12.38     6.19
## 2      4      Fe Dark  Dark  Fe 2463200.00    12.83     6.41
## 3      4      Fe Dark  Dark  Fe 2434650.00    12.98     6.49
## 4      4 Fe Light Light  Fe 2210200.00    21.63    10.81
## 5      4 Fe Light Light  Fe 2655700.00    18.00     9.00
## 6      4 Fe Light Light  Fe 2506350.00    19.07     9.54
```

```
data$Time <- as.factor(data$Time)
data$lum.cell2 <-as.numeric(as.character(data$lum.cell2))
```

...

```

lum4=subset(data, Time=="4")
lum11=subset(data, Time=="11")
lum20=subset(data, Time=="20")
lum26=subset(data, Time=="26")
lum30=subset(data, Time=="30")
lum33=subset(data, Time=="33")
lum48=subset(data, Time=="48")
lum57=subset(data, Time=="57")

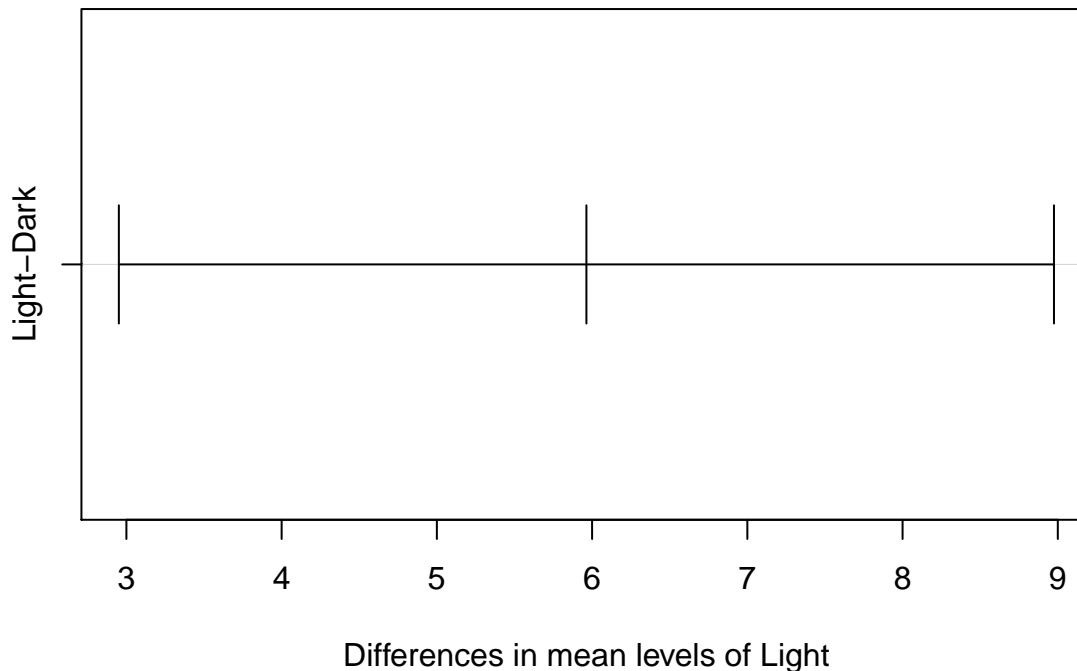
lum4.s<-aov(lum.cell2~Iron*Light,data=lum4)
summary(lum4.s)

##              Df Sum Sq Mean Sq F value    Pr(>F)
## Iron          1  27.24    27.24     5.322 0.04992 *
## Light         1 106.68   106.68    20.845 0.00184 **
## Iron:Light     1  19.41    19.41     3.792 0.08737 .
## Residuals     8  40.94     5.12
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Iron p=0.05004
#Light p=0.00184
#Iron:Light 0.08726
p=plot(TukeyHSD(lum4.s, "Light"))

```

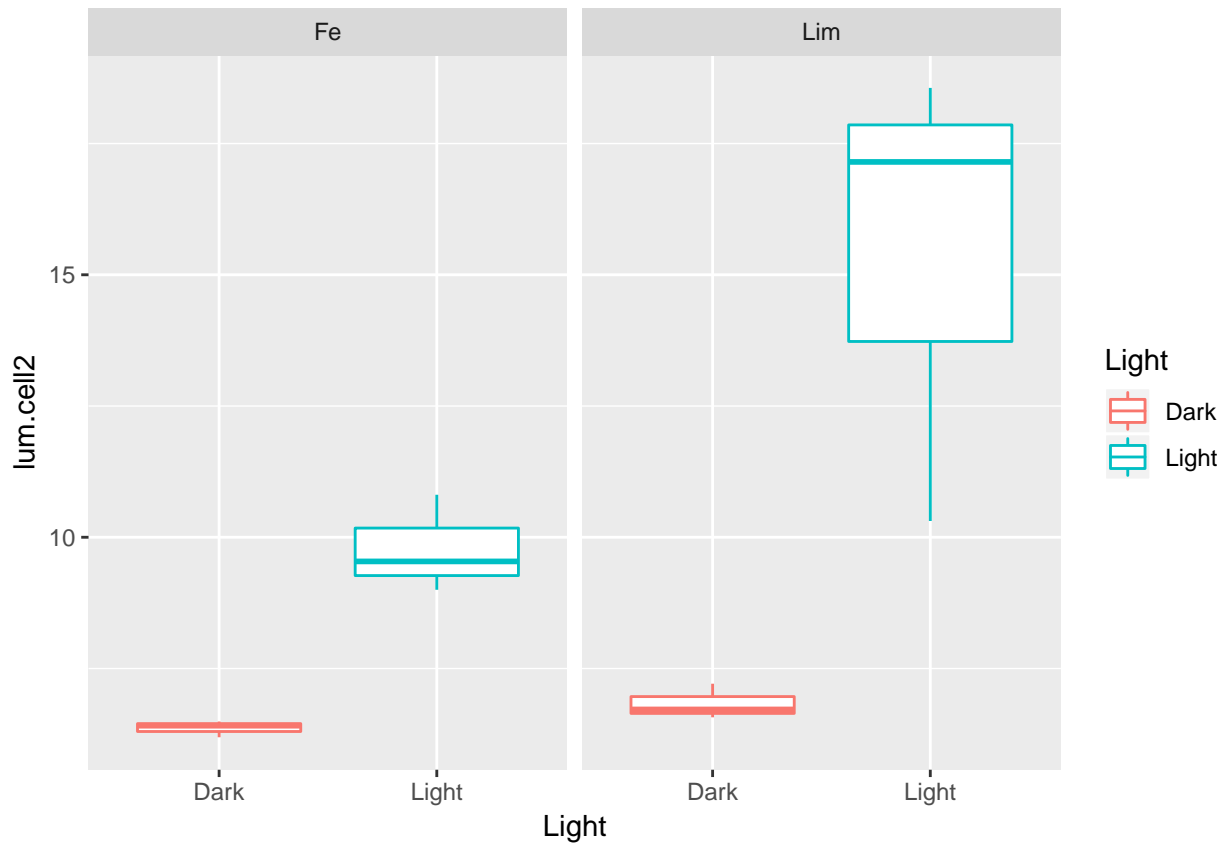
95% family-wise confidence level



p

NULL

```
ggplot(data=lum4, aes(x=Light, y=lum.cell2, colour=Light)) +geom_boxplot() +facet_grid(.~Iron)
```



```
shapiro.test(residuals(lum4.s)) #p=0.01841
```

```
##
## Shapiro-Wilk normality test
##
## data: residuals(lum4.s)
## W = 0.82513, p-value = 0.01835
```

```
leveneTest(lum4.s) #p=0.3207
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 3  1.3667 0.3208
##      8
```

```
TukeyHSD(lum4.s)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = lum.cell2 ~ Iron * Light, data = lum4)
##
## $Iron
##      diff      lwr      upr    p adj
## Lim-Fe 3.013333 0.001348515 6.025318 0.0499195
##
```

```
## $Light
##           diff      lwr      upr      p adj
## Light-Dark 5.963333 2.951349 8.975318 0.0018364
##
## $`Iron:Light`
##           diff      lwr      upr      p adj
## Lim:Dark-Fe:Dark 0.470000 -5.4452998 6.38530 0.9937584
## Fe:Light-Fe:Dark 3.420000 -2.4952998 9.33530 0.3188037
## Lim:Light-Fe:Dark 8.976667 3.0613669 14.89197 0.0054941
## Fe:Light-Lim:Dark 2.950000 -2.9652998 8.86530 0.4313750
## Lim:Light-Lim:Dark 8.506667 2.5913669 14.42197 0.0075568
## Lim:Light-Fe:Light 5.556667 -0.3586331 11.47197 0.0657481
#Fe:Light-Fe:Dark 3.4184425 -2.4994686 9.336354 0.3194617
#Lim:Light-Lim:Dark 8.5094563 2.5915452 14.427368 0.0075618

mean_name= ddpoly(lum4,.(Cond), #all different groupings required
                 summarize, meanvalue=mean(lum.cell2),
                 SE = sd(lum.cell2) / sqrt((length(lum.cell2))))
head(mean_name)

##           Cond meanvalue      SE
## 1 Fe Dark 6.363333 0.08969083
## 2 Fe Light 9.783333 0.53648030
## 3 Lim Dark 6.833333 0.19324711
## 4 Lim Light 15.340000 2.54772447

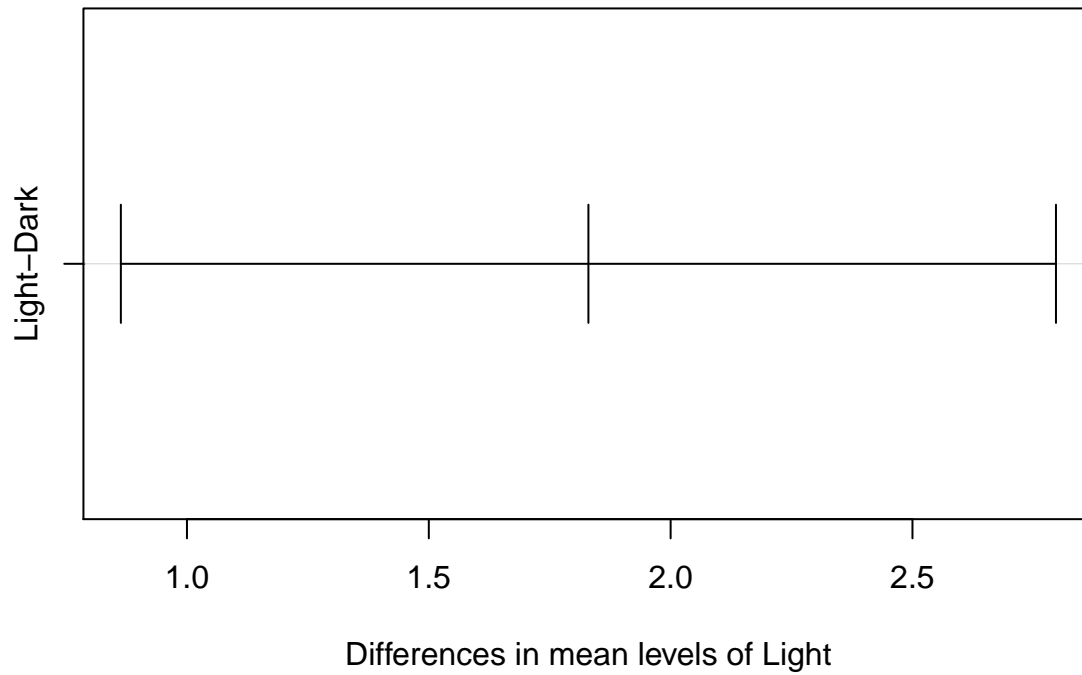
#####

lum11.s<-aov(lum.cell2~Iron*Light,data=lum11)
summary(lum11.s)

##           Df Sum Sq Mean Sq F value Pr(>F)
## Iron      1 629.6    629.6 1194.253 5.38e-10 ***
## Light     1  10.0     10.0   19.057  0.0024 **
## Iron:Light 1   2.5      2.5    4.817  0.0595 .
## Residuals 8   4.2      0.5
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Iron p=5.31*10 -10
#Light p=0.00236
#Iron:Light 0.05948
p=plot(TukeyHSD(lum11.s, "Light"))
```

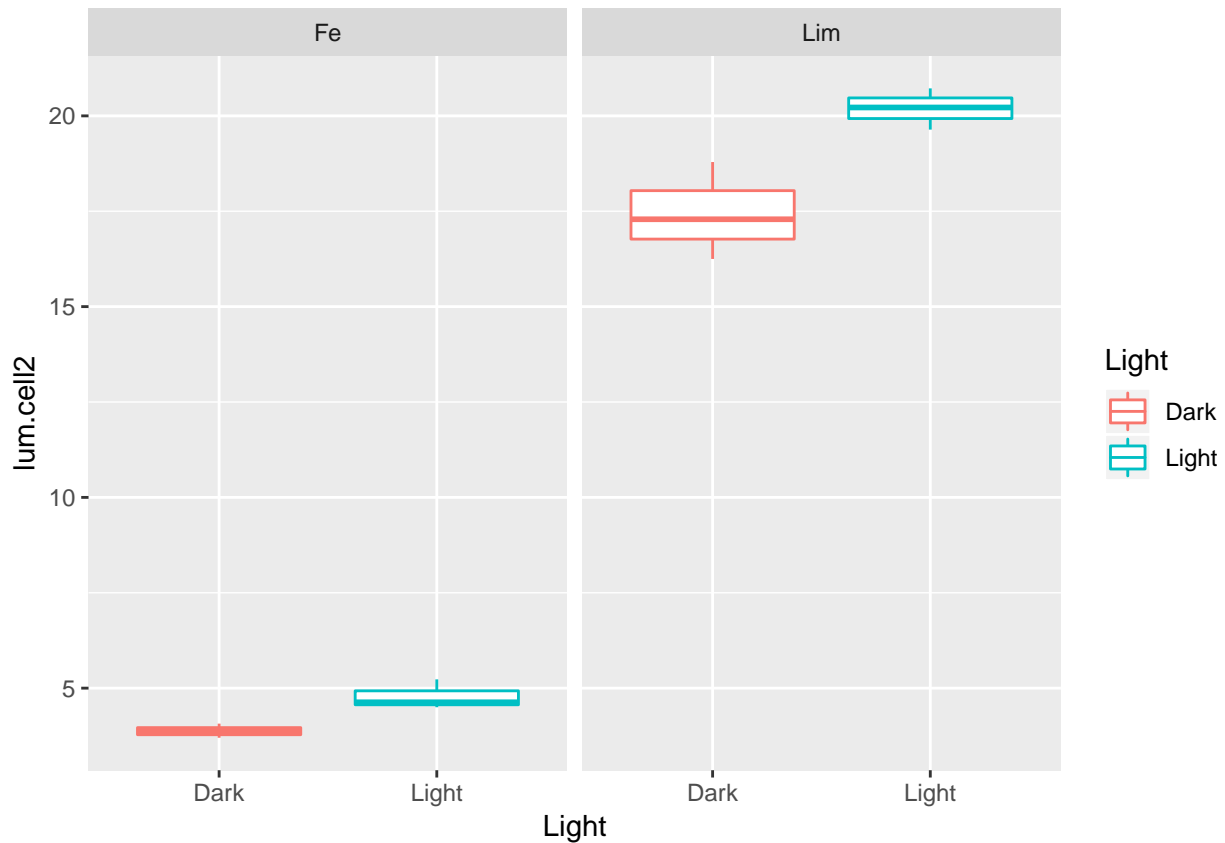
95% family-wise confidence level



p

NULL

```
ggplot(data=lum11, aes(x=Light, y=lum.cell12, colour=Light)) +geom_boxplot() +facet_grid(.~Iron)
```



```
shapiro.test(residuals(lum11.s)) #p=0.624
```

```
##
## Shapiro-Wilk normality test
##
## data: residuals(lum11.s)
## W = 0.94915, p-value = 0.6246
```

```
leveneTest(lum11.s) #p=0.2859
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 3  1.5082 0.2849
##      8
```

```
TukeyHSD(lum11.s)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = lum.cell2 ~ Iron * Light, data = lum11)
##
## $Iron
##      diff      lwr      upr p adj
## Lim-Fe 14.48667 13.51999 15.45334 0
##
## $Light
##      diff      lwr      upr p adj
## Light-Dark 1.83 0.8633254 2.796675 0.002395
```



```
##
## $`Iron:Light`
##           diff           lwr           upr           p adj
## Lim:Dark-Fe:Dark  13.56667  11.6681943  15.465139  0.0000000
## Fe:Light-Fe:Dark   0.91000  -0.9884723   2.808472  0.4621747
## Lim:Light-Fe:Dark  16.31667  14.4181943  18.215139  0.0000000
## Fe:Light-Lim:Dark -12.65667 -14.5551390 -10.758194  0.0000001
## Lim:Light-Lim:Dark  2.75000   0.8515277   4.648472  0.0072430
## Lim:Light-Fe:Light 15.40667  13.5081943  17.305139  0.0000000
#Fe:Light-Fe:Dark  0.9127591 -0.9823974  2.807916 0.4584656
#Lim:Light-Lim:Dark 2.7495790  0.8544225  4.644736 0.0071755

mean_name= ddpby(lum11,.(Cond), #all different groupings required
                summarize, meanvalue=mean(lum.cell2),
                SE = sd(lum.cell2) / sqrt((length(lum.cell2))))
head(mean_name)

##           Cond meanvalue           SE
## 1   Fe Dark  3.876667  0.1071344
## 2   Fe Light  4.786667  0.2248209
## 3   Lim Dark 17.443333  0.7372321
## 4   Lim Light 20.193333  0.3120541

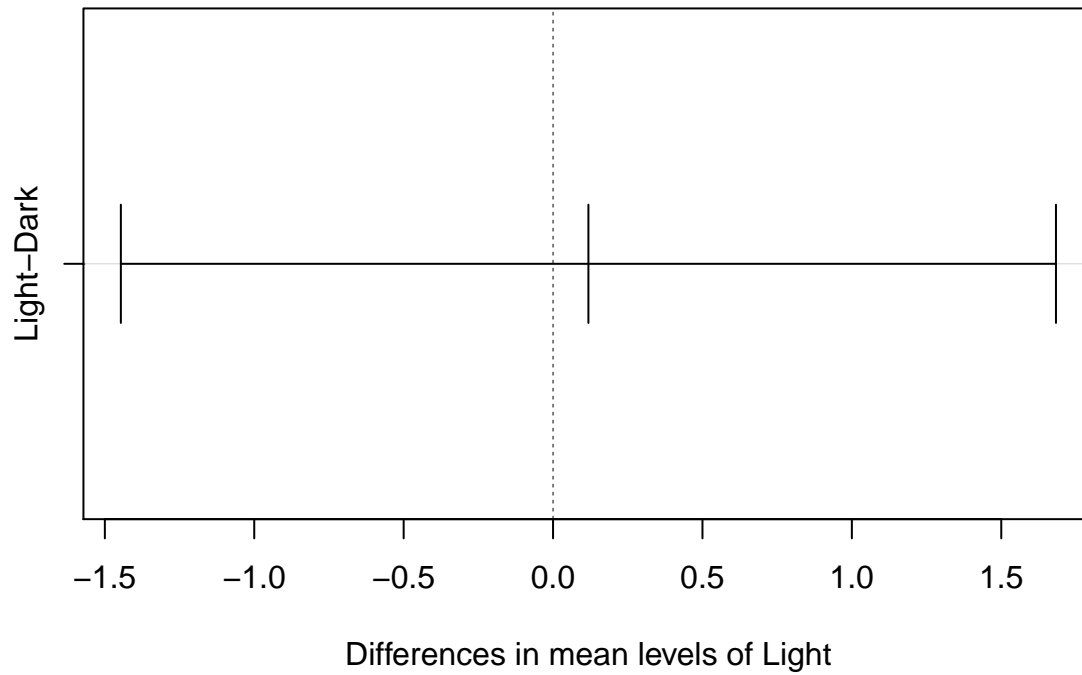
#####

lum20.s<-aov(lum.cell2~Iron*Light,data=lum20)
summary(lum20.s)

##           Df Sum Sq Mean Sq  F value    Pr(>F)
## Iron           1 1510.4  1510.4  1093.449 7.63e-10 ***
## Light           1   0.0    0.0    0.030   0.866
## Iron:Light       1   0.1    0.1    0.042   0.844
## Residuals       8   11.1    1.4
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Iron p=7.6 *10e-10
#Light p=0.864
#Iron:Light 0.845
p=plot(TukeyHSD(lum20.s, "Light"))
```

95% family-wise confidence level



p

NULL

```
shapiro.test(residuals(lum20.s)) #p=0.2804
```

##

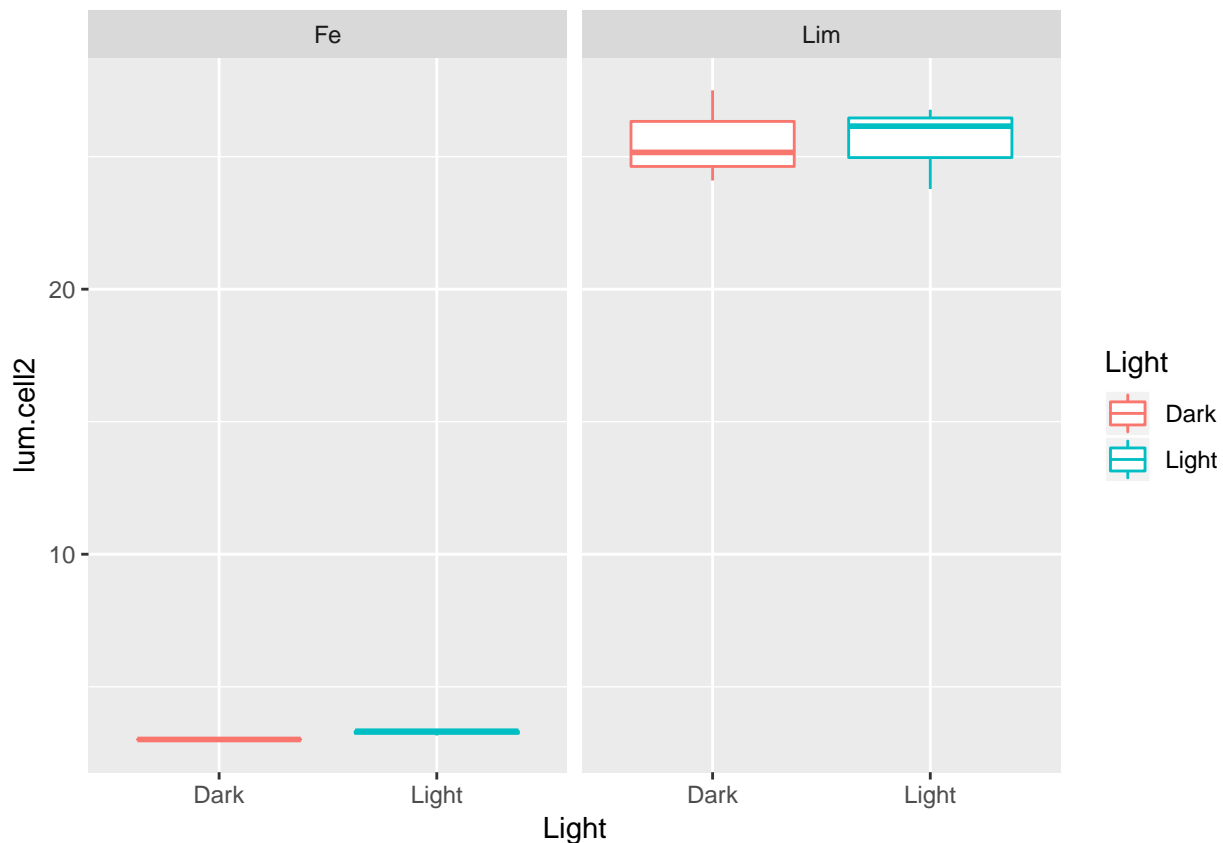
Shapiro-Wilk normality test

##

data: residuals(lum20.s)

W = 0.92004, p-value = 0.2862

```
ggplot(data=lum20, aes(x=Light, y=lum.cell12, colour=Light)) +geom_boxplot() +facet_grid(.~Iron)
```



```
leveneTest(lum20.s) #p=0.286
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 3   1.496 0.2878
##      8
```

```
TukeyHSD(lum20.s)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = lum.cell2 ~ Iron * Light, data = lum20)
##
## $Iron
##      diff      lwr      upr p adj
## Lim-Fe 22.43833 20.87356 24.00311    0
##
## $Light
##      diff      lwr      upr    p adj
## Light-Dark 0.1183333 -1.44644 1.683106 0.8658931
##
## $`Iron:Light`
##      diff      lwr      upr    p adj
## Lim:Dark-Fe:Dark 22.5766667 19.503576 25.649757 0.0000000
## Fe:Light-Fe:Dark 0.2566667 -2.816424 3.329757 0.9927753
## Lim:Light-Fe:Dark 22.5566667 19.483576 25.629757 0.0000000
## Fe:Light-Lim:Dark -22.3200000 -25.393090 -19.246910 0.0000000
```

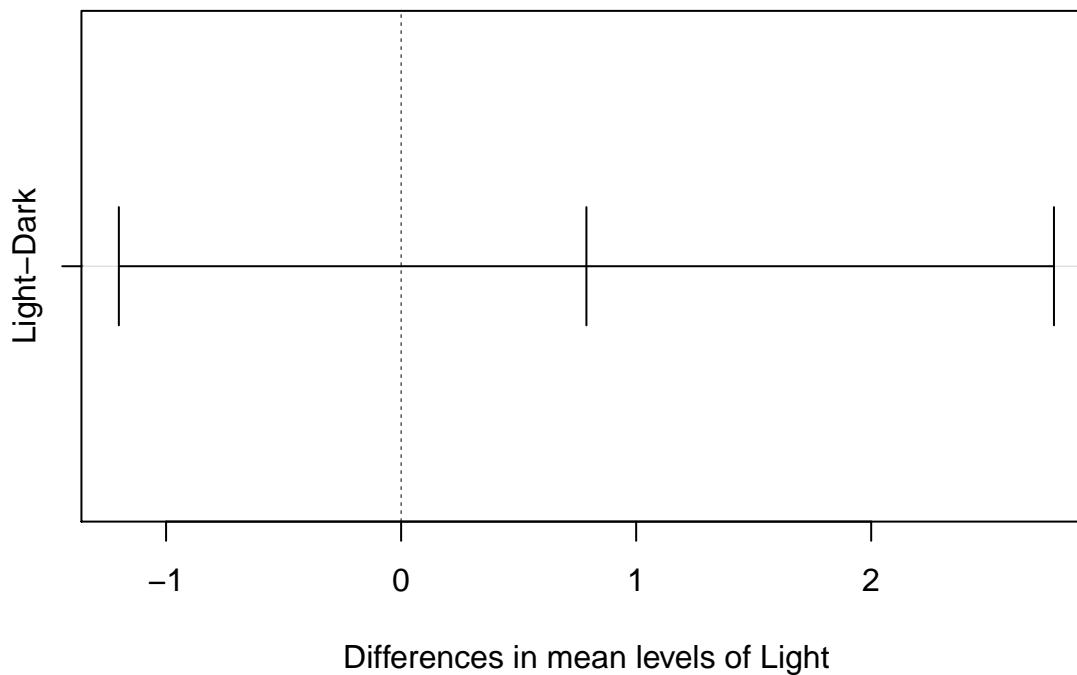
```
## Lim:Light-Lim:Dark -0.0200000 -3.093090 3.053090 0.9999965
## Lim:Light-Fe:Light 22.3000000 19.226910 25.373090 0.0000000
#Fe:Light-Fe:Dark 0.25692336 -2.814635 3.328482 0.9927436
#Lim:Light-Lim:Dark -0.01619445 -3.087753 3.055364 0.9999981
#####
```

```
lum26.s<-aov(lum.cell2~Iron*Light,data=lum26)
summary(lum26.s)
```

```
##          Df Sum Sq Mean Sq F value    Pr(>F)
## Iron          1    745.0    745.0 333.684 8.3e-08 ***
## Light         1     1.9     1.9   0.835   0.388
## Iron:Light     1     1.6     1.6   0.706   0.425
## Residuals     8    17.9     2.2
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#Iron p=8.38e-08
#Light p=0.386
#Iron:Light 0.426
p=plot(TukeyHSD(lum26.s, "Light"))
```

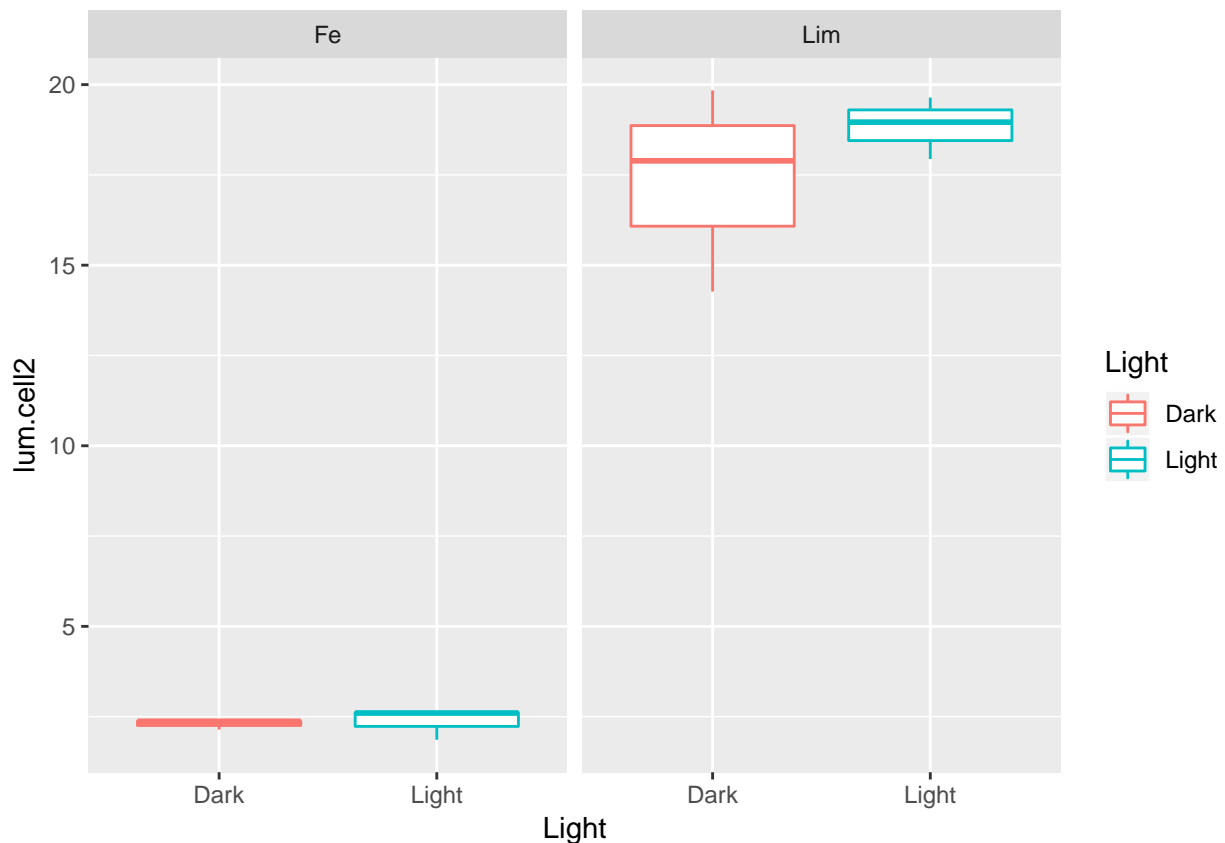
95% family-wise confidence level



p

```
## NULL
```

```
ggplot(data=lum26, aes(x=Light, y=lum.cell2, colour=Light)) +geom_boxplot() +facet_grid(.~Iron)
```



```
shapiro.test(residuals(lum26.s)) #p=0.06986
```

```
##
## Shapiro-Wilk normality test
##
## data: residuals(lum26.s)
## W = 0.87229, p-value = 0.06987
```

```
leveneTest(lum26.s) #p=0.1834
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 3  2.0642 0.1835
##      8
```

```
TukeyHSD(lum26.s)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = lum.cell2 ~ Iron * Light, data = lum26)
##
## $Iron
##      diff      lwr      upr p adj
## Lim-Fe 15.75833 13.76902 17.74764 1e-07
##
## $Light
##      diff      lwr      upr      p adj
## Light-Dark 0.7883333 -1.200977 2.777643 0.387516
```

```
##
## $`Iron:Light`
##               diff      lwr      upr      p adj
## Lim:Dark-Fe:Dark 15.03333333 11.126486 18.940181 0.0000083
## Fe:Light-Fe:Dark  0.06333333 -3.843514  3.970181 0.9999453
## Lim:Light-Fe:Dark 16.54666667 12.639819 20.453514 0.0000040
## Fe:Light-Lim:Dark -14.97000000 -18.876848 -11.063152 0.0000085
## Lim:Light-Lim:Dark  1.51333333 -2.393514  5.420181 0.6209684
## Lim:Light-Fe:Light 16.48333333 12.576486 20.390181 0.0000041
```

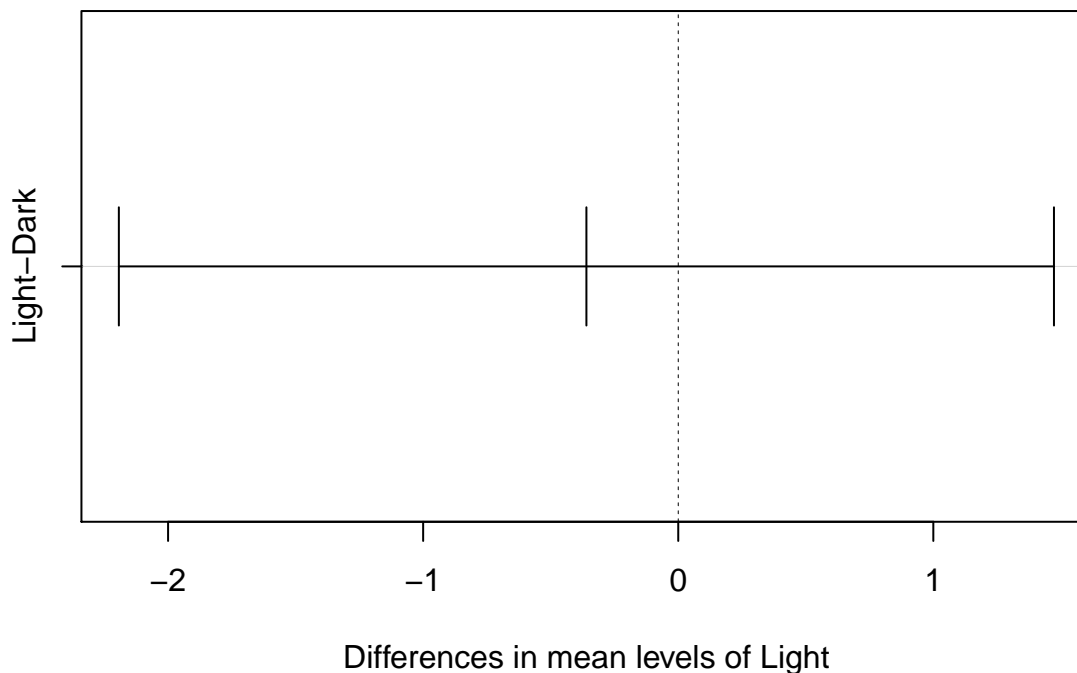
```
#Fe:Light-Fe:Dark 0.06717701 -3.845483 3.979837 0.9999350
#Lim:Light-Lim:Dark 1.51580818 -2.396852 5.428468 0.6208661
#####
```

```
lum30.s<-aov(lum.cell2~Iron*Light,data=lum30)
summary(lum30.s)
```

```
##           Df Sum Sq Mean Sq F value    Pr(>F)
## Iron          1 220.33   220.33 116.243 4.83e-06 ***
## Light         1   0.39    0.39   0.205  0.6626
## Iron:Light     1  15.69   15.69   8.276  0.0206 *
## Residuals     8  15.16    1.90
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#Iron p=4.86e-06
#Light p=0.6632
#Iron:Light 0.0207
p=plot(TukeyHSD(lum30.s, "Light"))
```

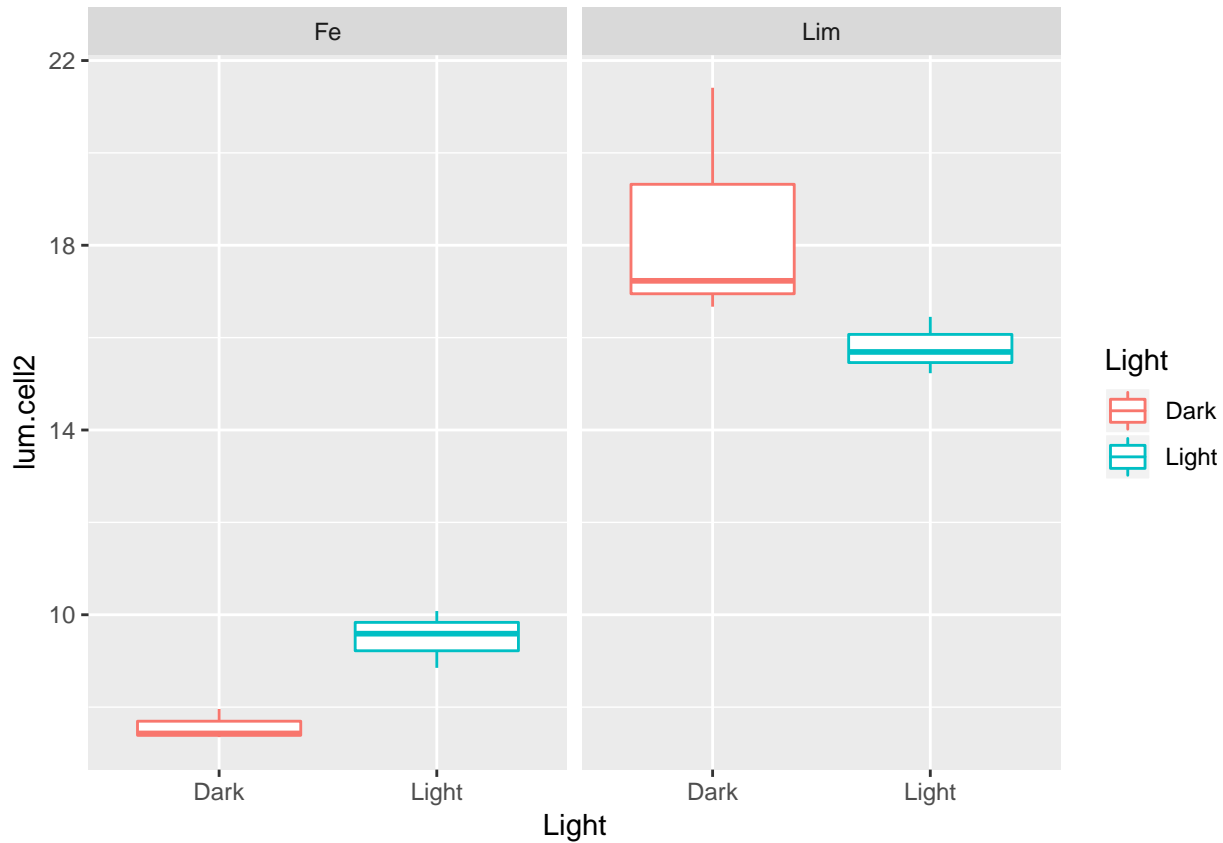
95% family-wise confidence level



p

```
## NULL
```

```
ggplot(data=lum30, aes(x=Light, y=lum.cell2, colour=Light)) +geom_boxplot() +facet_grid(.~Iron)
```



```
shapiro.test(residuals(lum30.s)) #p=0.1363
```

```
##  
## Shapiro-Wilk normality test  
##  
## data: residuals(lum30.s)  
## W = 0.89499, p-value = 0.1367
```

```
leveneTest(lum30.s) #p=0.5028
```

```
## Levene's Test for Homogeneity of Variance (center = median)  
##      Df F value Pr(>F)  
## group 3  0.8562 0.5017  
##      8
```

```
TukeyHSD(lum30.s)
```

```
## Tukey multiple comparisons of means  
## 95% family-wise confidence level  
##  
## Fit: aov(formula = lum.cell2 ~ Iron * Light, data = lum30)  
##  
## $Iron
```

```
##          diff      lwr      upr    p adj
## Lim-Fe 8.57 6.737021 10.40298 4.8e-06
##
## $Light
##          diff      lwr      upr    p adj
## Light-Dark -0.36 -2.192979 1.472979 0.6626493
##
## $`Iron:Light`
##          diff      lwr      upr    p adj
## Lim:Dark-Fe:Dark 10.856667 7.256841 14.4564927 0.0000512
## Fe:Light-Fe:Dark 1.926667 -1.673159 5.5264927 0.3767826
## Lim:Light-Fe:Dark 8.210000 4.610174 11.8098260 0.0003828
## Fe:Light-Lim:Dark -8.930000 -12.529826 -5.3301740 0.0002115
## Lim:Light-Lim:Dark -2.646667 -6.246493 0.9531594 0.1643854
## Lim:Light-Fe:Light 6.283333 2.683507 9.8831594 0.0023036
#Fe:Light-Fe:Dark 1.927439 -1.675256 5.5301343 0.3770858
#Lim:Light-Lim:Dark -2.646789 -6.249484 0.9559065 0.1647844
#####

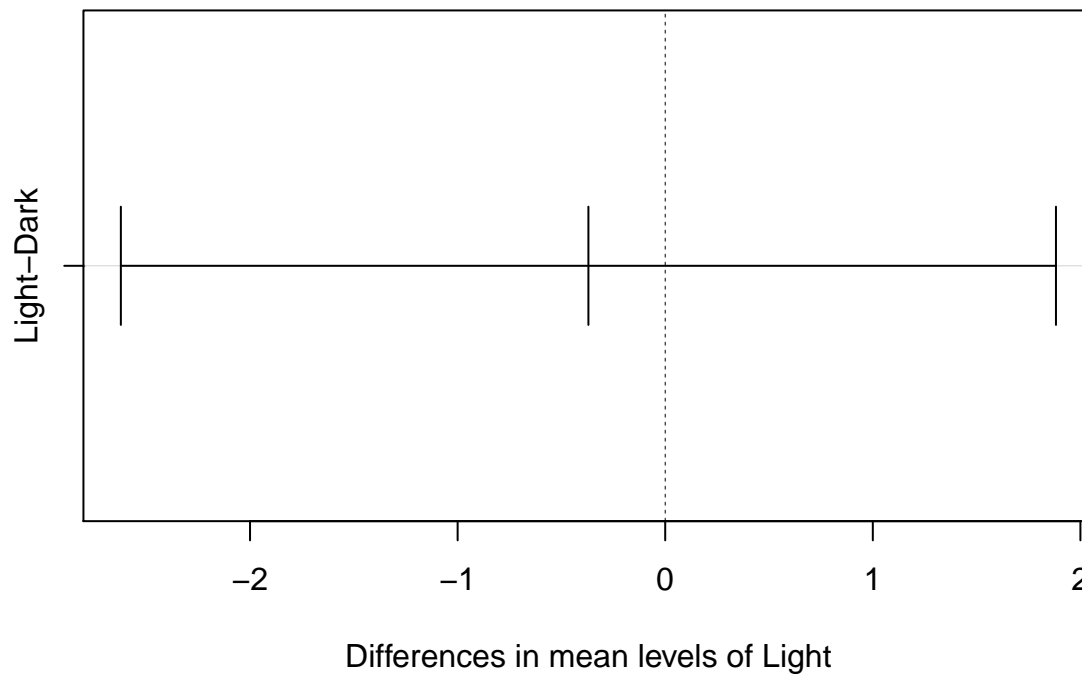
#####

lum33.s<-aov(lum.cell2~Iron*Light,data=lum33)
summary(lum33.s)

##          Df Sum Sq Mean Sq F value    Pr(>F)
## Iron          1  96.45    96.45   33.701 0.000403 ***
## Light          1   0.41     0.41    0.144 0.714674
## Iron:Light      1  25.87    25.87    9.040 0.016898 *
## Residuals      8  22.89     2.86
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Iron p=4.86e-06
#Light p=0.6632
#Iron:Light 0.0207
p=plot(TukeyHSD(lum33.s, "Light"))
```

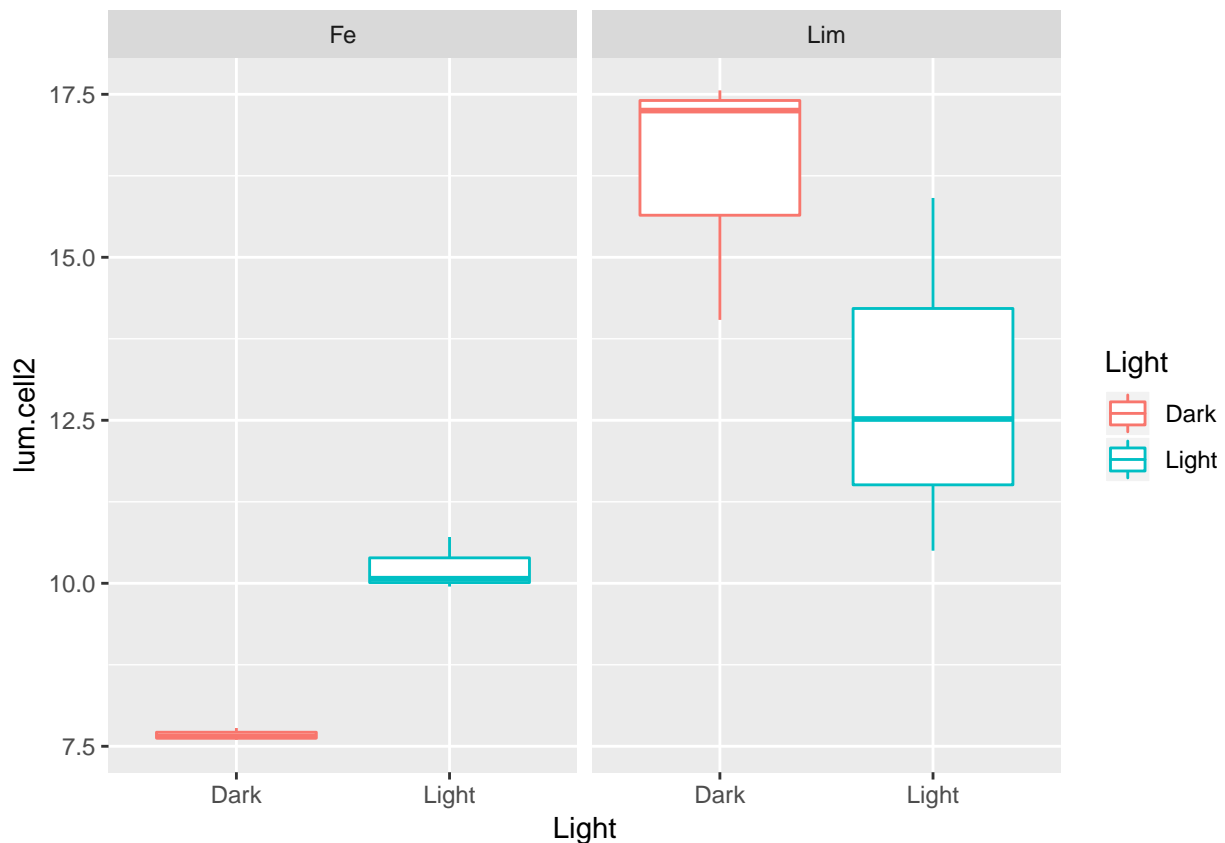

95% family-wise confidence level



p

NULL

```
ggplot(data=lum33, aes(x=Light, y=lum.cell12, colour=Light)) +geom_boxplot() +facet_grid(.~Iron)
```



```
shapiro.test(residuals(lum33.s)) #p=0.1363
```

```
##
## Shapiro-Wilk normality test
##
## data: residuals(lum33.s)
## W = 0.92984, p-value = 0.3784
```

```
leveneTest(lum33.s) #p=0.5028
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 3  1.2918 0.3419
##      8
```

```
TukeyHSD(lum33.s)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = lum.cell2 ~ Iron * Light, data = lum33)
##
## $Iron
##      diff      lwr      upr    p adj
## Lim-Fe 5.67 3.417714 7.922286 0.0004028
##
## $Light
##      diff      lwr      upr    p adj
## Light-Dark -0.37 -2.622286 1.882286 0.7146742
```

```
##
## $`Iron:Light`
##               diff               lwr               upr               p adj
## Lim:Dark-Fe:Dark    8.606667    4.1833554 13.029978 0.0011325
## Fe:Light-Fe:Dark    2.566667   -1.8566446  6.989978 0.3161515
## Lim:Light-Fe:Dark    5.300000    0.8766887  9.723311 0.0207500
## Fe:Light-Lim:Dark   -6.040000  -10.4633113 -1.616689 0.0101830
## Lim:Light-Lim:Dark  -3.306667   -7.7299780  1.116645 0.1556922
## Lim:Light-Fe:Light   2.733333   -1.6899780  7.156645 0.2713522
#Fe:Light-Fe:Dark    1.927439   -1.675256  5.5301343 0.3770858
#Lim:Light-Lim:Dark  -2.646789   -6.249484  0.9559065 0.1647844

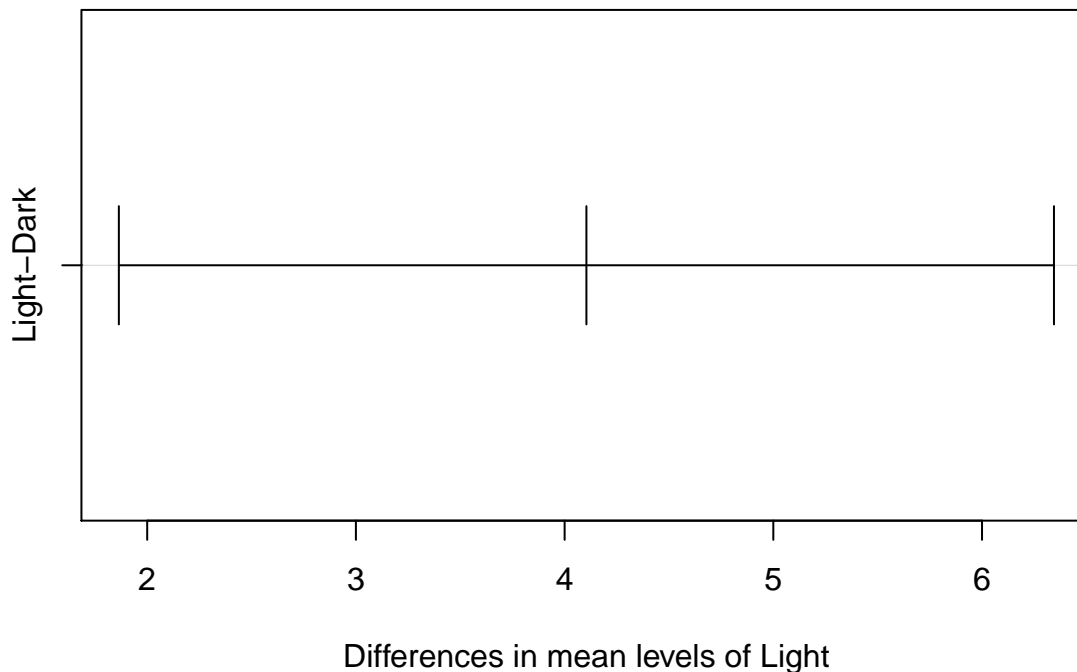
#####

lum48.s<-aov(lum.cell2~Iron*Light,data=lum48)
summary(lum48.s)

##              Df Sum Sq Mean Sq F value   Pr(>F)
## Iron           1  10.69   10.69    2.178 0.16217
## Light          1  75.81   75.81   15.440 0.00151 **
## Iron:Light      1  60.35   60.35   12.292 0.00349 **
## Residuals     14  68.74    4.91
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Iron p=0.16182
#Light p=0.00150
#Iron:Light 0.00349
p=plot(TukeyHSD(lum48.s, "Light"))
```

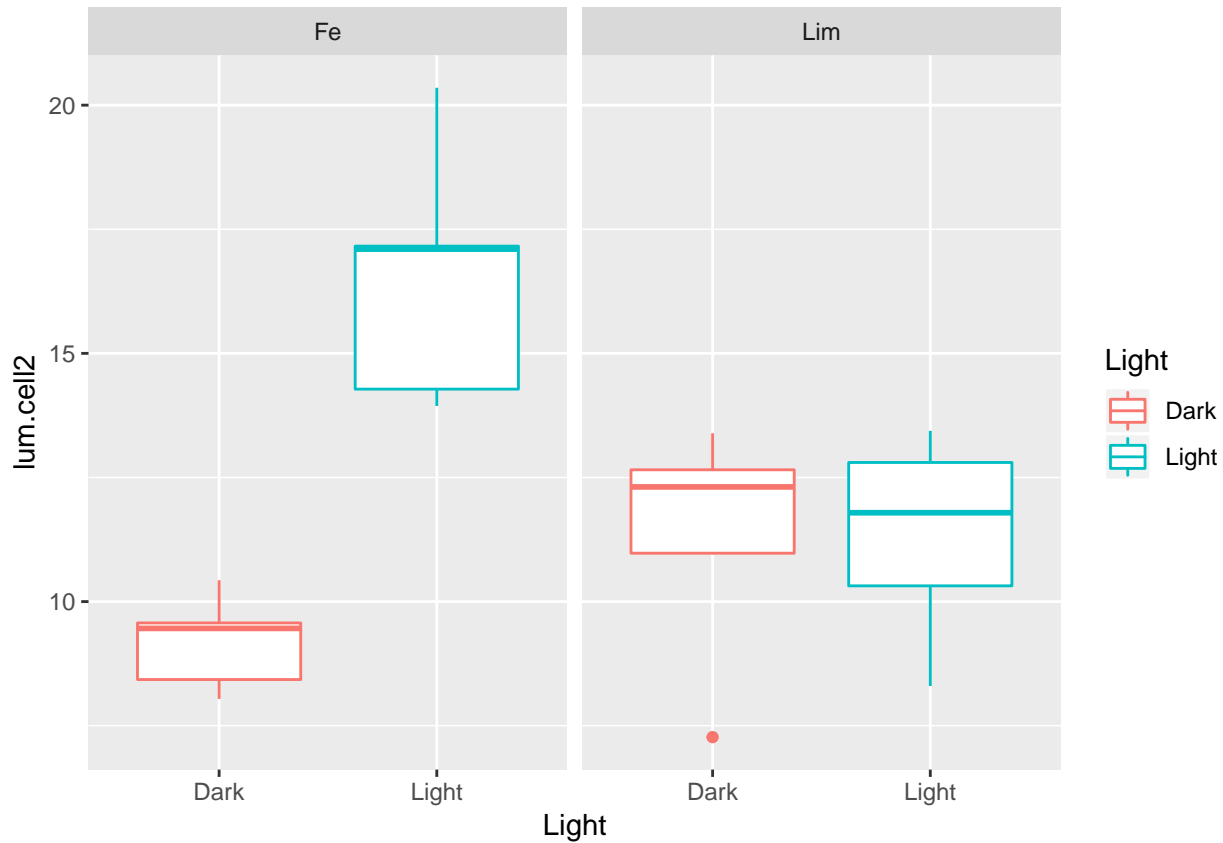
95% family-wise confidence level



p

```
## NULL
```

```
ggplot(data=lum48, aes(x=Light, y=lum.cell2, colour=Light)) +geom_boxplot() +facet_grid(.~Iron)
```



```
shapiro.test(residuals(lum48.s)) #p=0.6681
```

```
##  
## Shapiro-Wilk normality test  
##  
## data: residuals(lum48.s)  
## W = 0.96342, p-value = 0.6688
```

```
leveneTest(lum48.s) #p=0.671
```

```
## Levene's Test for Homogeneity of Variance (center = median)  
##      Df F value Pr(>F)  
## group 3  0.5301  0.669  
##      14
```

```
TukeyHSD(lum48.s)
```

```
## Tukey multiple comparisons of means  
## 95% family-wise confidence level  
##  
## Fit: aov(formula = lum.cell2 ~ Iron * Light, data = lum48)  
##  
## $Iron
```

```
##           diff      lwr      upr      p adj
## Lim-Fe -1.551 -3.805274 0.7032743 0.162167
##
## $Light
##           diff      lwr      upr      p adj
## Light-Dark 4.104444 1.864129 6.34476 0.0015117
##
## $`Iron:Light`
##           diff      lwr      upr      p adj
## Lim:Dark-Fe:Dark 2.134 -2.1863405 6.4543405 0.4991949
## Fe:Light-Fe:Dark 7.380 3.3067439 11.4532561 0.0006112
## Lim:Light-Fe:Dark 2.144 -2.1763405 6.4643405 0.4953889
## Fe:Light-Lim:Dark 5.246 0.9256595 9.5663405 0.0155830
## Lim:Light-Lim:Dark 0.010 -4.5440388 4.5640388 0.9999999
## Lim:Light-Fe:Light -5.236 -9.5563405 -0.9156595 0.0157831
#Fe:Light-Fe:Dark 7.38014721 3.3088569 11.4514375 0.0006083
#Lim:Light-Lim:Dark 0.01126531 -4.5405756 4.5631062 0.9999999

mean_name= ddpby(lum48,.(Cond), #all different groupings required
                summarize, meanvalue=mean(lum.cell2),
                SE = sd(lum.cell2) / sqrt((length(lum.cell2))))
head(mean_name)

##           Cond meanvalue      SE
## 1 Fe Dark 9.186 0.427488
## 2 Fe Light 16.566 1.163579
## 3 Lim Dark 11.320 1.374397
## 4 Lim Light 11.330 1.130494

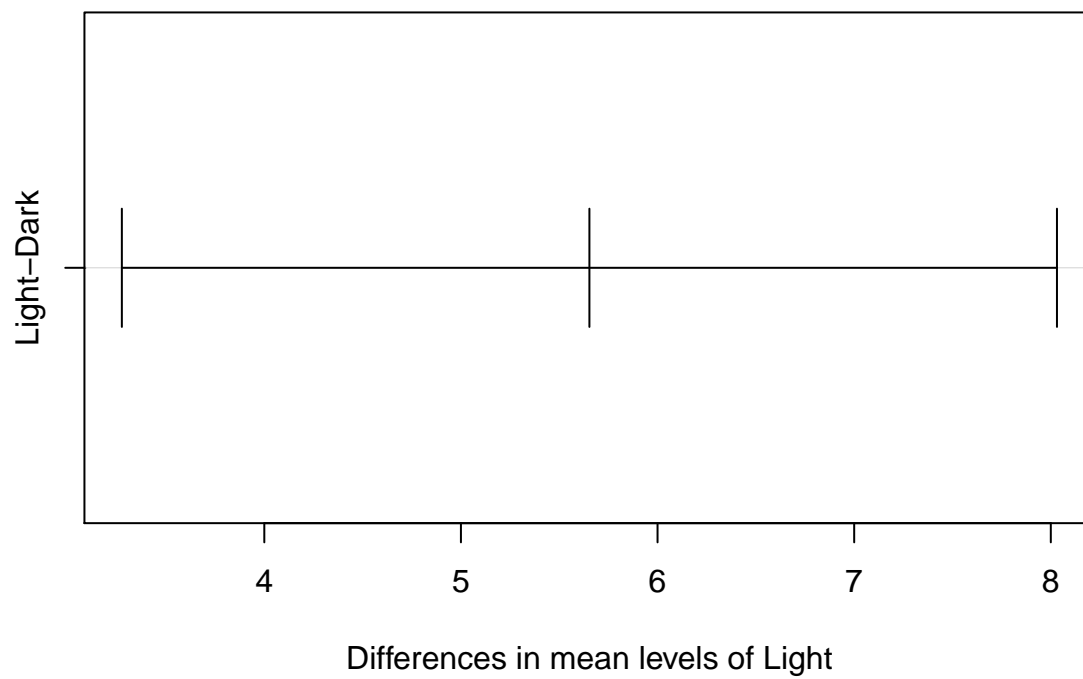
#####

lum57.s<-aov(lum.cell2~Iron*Light,data=lum57)
summary(lum57.s)

##           Df Sum Sq Mean Sq F value    Pr(>F)
## Iron        1 221.33  221.33    43.13 1.80e-05 ***
## Light        1 135.78  135.78    26.46 0.000189 ***
## Iron:Light    1 214.70  214.70    41.84 2.11e-05 ***
## Residuals   13  66.71    5.13
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Iron p=1.8e-05
#Light p=0.000189
#Iron:Light 2.1e-05
p=plot(TukeyHSD(lum57.s, "Light"))
```

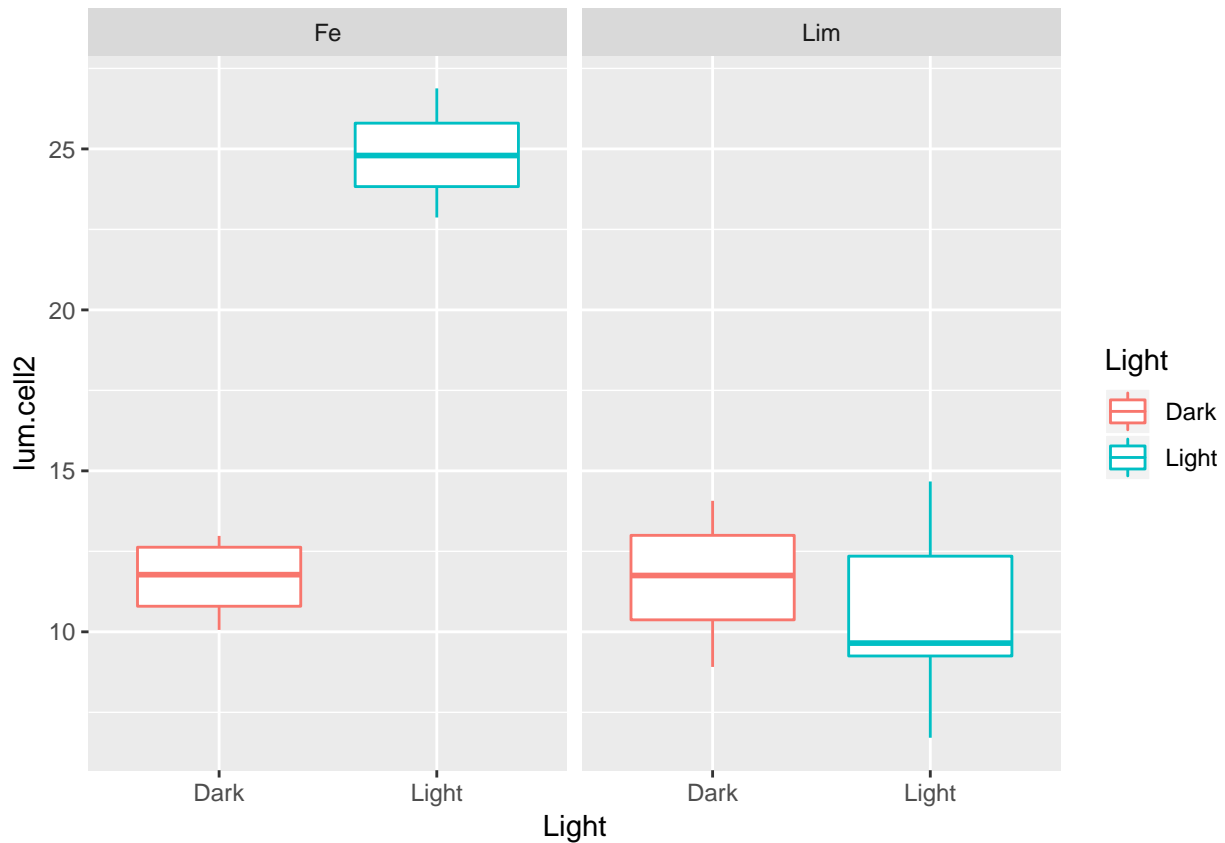
95% family-wise confidence level



p

NULL

```
ggplot(data=lum57, aes(x=Light, y=lum.cell12, colour=Light)) +geom_boxplot() +facet_grid(.~Iron)
```



```
shapiro.test(residuals(lum57.s)) #p=0.9934
```

```
##
## Shapiro-Wilk normality test
##
## data: residuals(lum57.s)
## W = 0.98637, p-value = 0.9935
```

```
leveneTest(lum57.s) #p=0.6078
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 3  0.6293 0.6089
##      13
```

```
TukeyHSD(lum57.s)
```

```
## Tukey multiple comparisons of means
## 95% family-wise confidence level
##
## Fit: aov(formula = lum.cell2 ~ Iron * Light, data = lum57)
##
## $Iron
##      diff      lwr      upr    p adj
## Lim-Fe -7.229028 -9.606997 -4.851058 1.8e-05
##
## $Light
##      diff      lwr      upr    p adj
## Light-Dark 5.653418 3.275449 8.031388 0.0001913
```

```
##
## $`Iron:Light`
##           diff           lwr           upr           p adj
## Lim:Dark-Fe:Dark   -0.0275   -4.728919   4.673919  0.9999981
## Fe:Light-Fe:Dark   13.1875    8.486081  17.888919  0.0000086
## Lim:Light-Fe:Dark  -1.1215   -5.581658   3.338658  0.8800531
## Fe:Light-Lim:Dark  13.2150    8.513581  17.916419  0.0000084
## Lim:Light-Lim:Dark -1.0940   -5.554158   3.366158  0.8874133
## Lim:Light-Fe:Light -14.3090  -18.769158  -9.848842  0.0000019
#Fe:Light-Fe:Dark   13.18770812   8.486226  17.889190  0.0000086
#Lim:Light-Fe:Light -14.31247611 -18.772694  -9.852258  0.0000019

mean_name= ddpby(lum57,.(Cond), #all different groupings required
               summarize, meanvalue=mean(lum.cell2),
               SE = sd(lum.cell2) / sqrt((length(lum.cell2))))
head(mean_name)

##           Cond meanvalue           SE
## 1   Fe Dark   11.6475  0.6713590
## 2   Fe Light   24.8350  0.8601599
## 3   Lim Dark   11.6200  1.1167139
## 4   Lim Light   10.5260  1.3685233
```