# Stats for LITE / light-induced transient effects

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In this document, statistical analysis of the differences observed in the data used to construct the figures are summarized. Statistical significances are denoted: '\*': p<0.05; '\*\*': p<0.01; '\*\*\*': p<0.001; '\*\*\*\*': p<0.0001; 'n.s.' = not significant.

Note that timepoints of stimulation of the experiments were dependent on establishing a stable baseline and therefore, these timepoints may vary from experiment to experiment. That is reflected in the choices of timepoints on which the actual test statistics were based.

Before starting, we define some functions to extract data rows from the data frames. Every column is a cell, and rows represent the data points in the timelapse. To extract the lifetimes of all cells at a timepoint:

```
ExtractRowIntoArray <- function(df, time){
  timeStep <- as.numeric(df[2,1]-df[1,1])  # Determine the timestep of the timelapse series
  rowNr <- round(time/timeStep)  # Calculate at which row these data are
  nCol <- as.numeric(ncol(df))
  Out<-as.numeric(df[rowNr,2:nCol] )  #extract the data into a numeric array
  return(Out)
}</pre>
```

To quantify decay rates, which is necessary when comparing data taken with dissimilar setups to avoid small systematic differences in calculated lifetimes:

```
GetDecay <-function(df, time1, time2){
  pre <- ExtractRowIntoArray(df, time1)
  post <- ExtractRowIntoArray(df, time2)
  diff <- pre - post
  return(diff)
}</pre>
```

To get out descriptive statistics and compare significance with Wilcoxon unpaired test:

```
Stats <- function(array1, array2){</pre>
 if (missing(array1) || missing(array2)) {
   stop("Both arguments must be supplied.")
 cat("Mean for: array1 ", mean(array1), " for: array2 ", mean(array2), "\n")
 cat("Var for: array1 ", var(array1), "
                                            for: array2 ", var(array2), "\n")
 # Perform Wilcoxon rank-sum test
 wilcox_test_result <- wilcox.test(array1, array2)</pre>
 w <- wilcox_test_result$p.value</pre>
 p = "data do not differ significantly"
 if (w < 0.00001){
   p = "significance = ***** "
 else if (w < 0.0001){
   p = "significance = **** "
 else if (w < 0.001){</pre>
   p = "significance = *** "
 else if (w < 0.01){</pre>
   p = "significance = ** "
 else if (w < 0.05){
   p = "significance = * "
  return(list(Wilcox = wilcox_test_result, P = p))
}
```

For testing more than one condition, e.g. no pretreatment VS 2 min VS 4 min pretreatment, we use the Kruskal-Wallis test, which is also parameter-free:

```
StatsKruskal <- function(list_of_arrays){</pre>
  #function expects a list of data arrays and will determine mean and variance of these array
s, as well as do a K-W test
  if (length(list_of_arrays)<3){</pre>
    stop("list of minimally 3 arrays required for Kruskal-Wallis test")
  for (array in list_of_arrays){
    cat("Mean ", mean(array),"
                                       Var ", var(array), "\n")
  # Perform kruskal-wallis test
  kruskal_test_result <- kruskal.test(list_of_arrays)</pre>
  w <- kruskal_test_result$p.value</pre>
  p = "data do not differ significantly"
  if (w < 0.00001){
    p = "significance = ***** "
  else if (w < 0.0001){</pre>
    p = "significance = **** "
  else if (w < 0.001){
    p = "significance = *** "
  else if (w < 0.01){
    p = "significance = ** "
  else if (w < 0.05){
    p = "significance = * "
  p = paste("====>>>>>>>
                              ",p," <<<<<<=====""
  return(list(kruskal = kruskal test result, P = p))
}
```

Finally, we set a common working directory for data files.

```
wd = 'D:\\LocalSurfDrive\\FF\\Figures_data\\INPUT_CSVS_FINAL\\' #input data files are suppos
ed to be in this folder.
```

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# Figure 1. Test for transientness of timelapse signals.

Each trace consists of a series of lifetime values. For transient signals, the values just after stimulation are higher than at a later timepoint:

#### For HeLa cells:

Compare the data of Fig. 1A (fdFLIM) to those of Fig. 1B (TCSPC). Stimulation data are taken at t = 135 s and return data are at y = 475 s in A, and at 150 s and 490 s in B. Tested is by what amount the lifetime has decayed over the indicated period, i.e. the teststatistic is the (lifetimes at 135 s - those at 475).

```
FigA <- paste(wd,"Fd_HeLa_Normal_light_Fig1A.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
FigB <- paste(wd,"1_HeLaH201_40nMIP_sustained_confocal_option2_Fig1B.csv",sep="") # get data
df_B <- read.csv(FigB, sep = ',')

A <- GetDecay(df_A, 135, 475)
B <- GetDecay(df_B, 150, 490)

S <- Stats(A, B)</pre>
```

```
## Mean for: array1 0.5628598 for: array2 0.150341
## Var for: array1 0.01589244 for: array2 0.01902816
```

```
print(S$Wilcox)
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 8170, p-value = 5.435e-13
## alternative hypothesis: true location shift is not equal to 0
```

```
print(S$P)
```

```
## [1] "=====>>>>>> significance = ***** <<<<<======"
```

#### For COS7 cells:

Compare the data of Fig. 1C (fdFLIM) to those of Fig. 1D (TCSPC). Stimulation data are taken at t = 200 s and return data are at y = 500 s in A, and at 175 s and 800 s in B. Teststatistic: decay of signal over the indicated timespan. Note that in D, even after extended time, 625 s after stimulation, the TCSPC data did not decay.

```
FigA <- paste(wd,"Fd_Cos7H250_transient_plotted_till_900s_Fig1C.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
FigB <- paste(wd,"1_Cos7H250_40nMIP_sustained_confocal_Fig1D.csv",sep="") # get data
df_B <- read.csv(FigB, sep = ',')

A <- GetDecay(df_A, 200, 500)
B <- GetDecay(df_B, 175, 800)

S <- Stats(A, B)</pre>
```

```
## Mean for: array1 0.09672833 for: array2 -0.02992211
## Var for: array1 0.005866427 for: array2 0.0004565453
```

```
print(S$Wilcox)
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 18761, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0</pre>
```

```
print(S$P)
```

```
## [1] "====>>>>>> significance = ***** <<<<<======"
```

# Figure 2. Test for differences in lifetimes upon shift to adjacent FOV.

Test statistic is the lifetime at the end of the traces in the upper graph, versus the beginning of the traces taken just after shifting the FOV (lower graph).

```
FigA <- paste(wd,"Fd_HeLa_shift_exp_part1_Fig2.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
FigB <- paste(wd,"Fd_HeLa_shift_exp_part2_Fig2.csv",sep="") # get data
df_B <- read.csv(FigB, sep = ',')

A <- ExtractRowIntoArray(df_A, 230)
B <- ExtractRowIntoArray(df_B, 5)

S <- Stats(A, B)</pre>
```

```
## Mean for: array1 2.326711 for: array2 3.083313
## Var for: array1 0.01475247 for: array2 0.003001685
```

```
print(S$Wilcox)
```

```
##
## Wilcoxon rank sum exact test
##
## data: array1 and array2
## W = 0, p-value = 5.956e-11
## alternative hypothesis: true location shift is not equal to 0
```

```
print(S$P)
```

```
## [1] "=====>>>>>> significance = ***** <<<<<======"
```

### Figure 3. Test for differences in transientness.

#### Panel A versus B, HeLa cells:

Compare the data of Fig. 3A (fdFLIM) to those of Fig. 3B (fdFLIM with ND filter). Stimulation data are taken at t = 50 s and return data are taken 100 s later in A, and at 115 s with return values 100 s later in B. Teststatistic: decay of signal over the indicated timespan.

```
FigA <- paste(wd,"Fd_HeLaH201_transient_Fig3A.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
FigB <- paste(wd,"Fd_HeLa_Low light_trial2_Fig3B.csv",sep="") # get data
df_B <- read.csv(FigB, sep = ',')

A <- GetDecay(df_A, 50, 150)
B <- GetDecay(df_B, 115, 215)

S <- Stats(A, B)</pre>
```

```
## Mean for: array1 0.5802555 for: array2 0.06334511
## Var for: array1 0.02422123 for: array2 0.01336506
```

```
print(S$Wilcox)
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 1796, p-value = 9.666e-14
## alternative hypothesis: true location shift is not equal to 0
```

```
print(S$P)
```

```
## [1] "=====>>>>>> significance = ***** <<<<<====="
```

### Panel C versus D, COS7 cells:

Compare the data of Fig. 3C (fdFLIM) to those of Fig. 3D (fdFLIM with ND filter). Stimulation data are taken at t = 170 s and return data are taken 800 s later in C, and at 240 s with return values 800 s later in D. Teststatistic: decay of signal over the indicated timespan.

```
FigA <- paste(wd,"Fd_Cos7_Normal_light_0.77_multiplied_Fig3C.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
FigB <- paste(wd,"Fd_Cos7_Low_light_Fig3D.csv",sep="") # get data
df_B <- read.csv(FigB, sep = ',')

A <- 0.77 * GetDecay(df_A, 170, 970) # note the one-time correction factor of 0.77 for calibr
ation mistake, see Results section.
B <- GetDecay(df_B, 240, 1040)

S <- Stats(A, B)</pre>
```

```
## Mean for: array1 0.1707775 for: array2 0.60554
## Var for: array1 0.008313078 for: array2 0.104474

print(S$Wilcox)
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 1963, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0</pre>
```

```
print(S$P)
```

```
## [1] "====>>>>>> significance = ***** <<<<<====="
```

Please note that a p value = 2.2 e-16, which occurs several times in our tests, in R is convention to indicate that it is extremely small.

### Panel E versus F, COS7 cells, with/without LED illumination:

Compare the data of Fig. 3E (TCSPC) to those of Fig. 3F (TCSPC with LED illumination). Stimulation data are taken at t = 240 s and return data are taken 500 s later in E, and at 200 s with return values 500 s later in F. Teststatistic: decay of signal over the indicated timespan.

```
FigA <- paste(wd,"1_Cos7H250_40nMIP_sustained_confocal_Fig3E.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
FigB <- paste(wd,"4_Cos7H250_40nMIP_transient_confocal_option_3_Fig3F.csv",sep="") # get data
df_B <- read.csv(FigB, sep = ',')

A <- GetDecay(df_A, 240, 740)
B <- GetDecay(df_B, 200, 700)

S <- Stats(A, B)</pre>
```

```
## Mean for: array1 0.009542691 for: array2 0.7517311
## Var for: array1 0.0004993614 for: array2 0.003570755
```

```
print(S$Wilcox)
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 5, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0</pre>
```

```
print(S$P)
```

```
## [1] "====>>>>>> significance = ***** <<<<<====="
```

## Figure 5D. Comparison of rescue by ascorbic acid:

Data in the presence of a.a. are compared to control, i.e. the data of Figure 3F. Teststatistic: decay of signal over the indicated timespan. Data from both 3F and 5D are taken at 200 s and 500 s later.

```
FigA <- paste(wd, "4_Cos7H250_40nMIP_transient_confocal_option_3_Fig3F.csv", sep="") # get data
df A <- read.csv(FigA, sep = ',')</pre>
FigB <- paste(wd, "8_Cos7H250_40nMIP_sustained_AA_rescue_confocal_option2_Fig5D.csv", sep="") #
df_B <- read.csv(FigB, sep = ',')</pre>
A <- GetDecay(df_A, 200, 700)
B <- GetDecay(df_B, 200, 700)
S <- Stats(A, B)
## Mean for: array1 0.7517311
                                      for: array2 0.009667909
## Var for: array1 0.003570755
                                         for: array2 0.0004493405
print(S$Wilcox)
##
   Wilcoxon rank sum test with continuity correction
##
##
## data: array1 and array2
## W = 240378, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0
print(S$P)
## [1] "====>>>>> significance = ***** <<<<<====="
```

## Figure 6. Decay rates under various conditions.

### Figure 6A:

Following uncaging of cAMP, lifetime values decay from a level that depends on the previous baseline. For fair comparison, because breakdown is exponential, we compare decay speed starting at identical lifetimes within the cAMP decay curve, i.e. we test significance between peak 1 Vs 2, peak 2 vs 3 and peak 1 vs 3 at timepoints 55 s, 355 s and 786 s for peak 1, 2 and 3, respectively. Note that correction for multiple testing was not necessary because as expected, these data are not significantly different.

```
FigA <-paste(wd,"10_caged_experiment_data_Fig6A.csv",sep="") # get data</pre>
 df_A <- read.csv(FigA, sep = ',')</pre>
A <- GetDecay(df_A, 65, 165)
 B <- GetDecay(df_A, 380, 480)
C <- GetDecay(df_A, 800, 900)</pre>
 S <- Stats(A, B)
## Mean for: array1 0.1801871
                                      for: array2 0.1852731
## Var for: array1 0.001433976
                                       for: array2 0.001145625
 print(S$Wilcox)
##
  Wilcoxon rank sum test with continuity correction
##
##
## data: array1 and array2
## W = 2462, p-value = 0.4292
## alternative hypothesis: true location shift is not equal to \theta
 print(S$P)
## [1] "====>>>>>> data do not differ significantly <<<<<<====="
 S <- Stats(A, C)
## Mean for: array1 0.1801871
                                      for: array2 0.1845844
## Var for: array1 0.001433976
                                       for: array2 0.001240754
 print(S$Wilcox)
##
  Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 2524, p-value = 0.5837
## alternative hypothesis: true location shift is not equal to \theta
 print(S$P)
## [1] "====>>>>>>
                        data do not differ significantly
                                                              <<<<<<======"
 S <- Stats(B, C)
```

```
## Mean for: array1 0.1852731 for: array2 0.1845844
## Var for: array1 0.001145625 for: array2 0.001240754
```

```
print(S$Wilcox)
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 2724, p-value = 0.8174
## alternative hypothesis: true location shift is not equal to 0
```

```
print(S$P)
```

```
## [1] "====>>>>>> data do not differ significantly <<<<<====="
```

# Figure 6B. Comparison of the effect of blue light given before or during stimulation with isoproterenol:

Blue light given before IsoP does not affect cAMP generation. Increase in lifetime is detected as the difference of baseline values at t = 50 and stimulated values at t = 500, as compared to control data taken from Fig. 3E at t = 100 s and t = 550 s. Teststatistic: increase in signal upon IsoP administration.

```
FigA <- paste(wd,"1_Cos7H250_40nMIP_sustained_confocal_Fig3E.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
FigB <- paste(wd,"10_Cos7H250_40nMIP_shining_light_before_stimulation_Fig6B.csv",sep="") # ge
t data
df_B <- read.csv(FigB, sep = ',')

A <- GetDecay(df_A, 100, 550)
B <- GetDecay(df_B, 50, 500)

S <- Stats(A, B)</pre>
```

```
## Mean for: array1 -0.8673886 for: array2 -0.7825448
## Var for: array1 0.004172273 for: array2 0.003477725
```

```
print(S$Wilcox)
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 41338, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0</pre>
```

```
print(S$P)
```

```
## [1] "====>>>>>> significance = ***** <<<<<====="
```

## Figure 7. Different agonists.

## Panel A versus B: Norepinephrin with and without LED illumination:

Drop in lifetime is detected as the difference of values at t = 230 and 500 s later. Teststatistic: decay of signal after stimulation.

```
FigA <- paste(wd,"11_Cos7H250_200nMNE_in_FB_only_Fig7A.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
FigB <- paste(wd,"11_Cos7H250_200nMNE_in_FB_LED_2mins_Fig7B.csv",sep="") # get data
df_B <- read.csv(FigB, sep = ',')

A <- GetDecay(df_A, 230, 730)
B <- GetDecay(df_B, 230, 730)
S <- Stats(A, B)</pre>
```

```
## Mean for: array1 0.2063475 for: array2 0.6058071
## Var for: array1 0.002263479 for: array2 0.005661956
```

```
print(S$Wilcox)
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 18, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0</pre>
```

```
print(S$P)
```

```
## [1] "=====>>>>>> significance = ***** <<<<<====="
```

#### Panel C versus D: Adrenalin with and without LED illumination:

Drop in lifetime is detected as the difference of values at t = 200 and 600 s later. Teststatistic: decay of signal after stimulation.

```
FigA <- paste(wd,"11_Cos7H250_250nMAdre_in_FB_only_Fig7C.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
FigB <- paste(wd,"11_Cos7H250_250nMAdre_in_FB_LED_2mins_Fig7D.csv",sep="") # get data
df_B <- read.csv(FigB, sep = ',')

A <- GetDecay(df_A, 200, 800)
B <- GetDecay(df_B, 200, 800)

S <- Stats(A, B)</pre>
```

```
## Mean for: array1 0.04289268 for: array2 0.714335
## Var for: array1 0.003814529 for: array2 0.001846706
```

```
print(S$Wilcox)
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 0, p-value < 2.2e-16
## alternative hypothesis: true location shift is not equal to 0</pre>
```

```
print(S$P)
```

```
## [1] "=====>>>>>> significance = ***** <<<<<======"
```

# Panel E versus F: Prostaglandin with and without LED illumination.

Drop in lifetime is detected as the difference of values at t = 240 and 500 s later. Teststatistic: decay of signal after stimulation.

```
FigA <- paste(wd,"11_Cos7H250_200nMPGE-1_in_FB_only_Fig7E.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
FigB <- paste(wd,"11_Cos7H250_200nMPGE-1_in_FB_LED_2mins_Fig7F.csv",sep="") # get data
df_B <- read.csv(FigB, sep = ',')

A <- GetDecay(df_A, 240, 740)
B <- GetDecay(df_B, 240, 740)

S <- Stats(A, B)</pre>
```

```
## Mean for: array1 0.1733938 for: array2 0.1718793
## Var for: array1 0.002606596 for: array2 0.002635679
```

```
print(S$Wilcox)
```

```
##
## Wilcoxon rank sum test with continuity correction
##
## data: array1 and array2
## W = 280563, p-value = 0.4982
## alternative hypothesis: true location shift is not equal to 0
```

### Figure 8. Control versus pre-illuminated agonists.

# Panel A vs B vs C: Isoproterenol with 0 s (control), 2 min or 6 min of LED illumination:

Absolute lifetimes at t = 300 are compared, noting that between these experiments, stimulation was at identical timepoints. Teststatistic: lifetime value.

```
FigA <- paste(wd, "12_Cos7H250_40nMIP_degradation_0mins_eppie_Fig8A.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')
A <- ExtractRowIntoArray(df_A, 300)
dataList <- list(array1 = A)

FigA <- paste(wd, "T0479_12_Cos7H250_40nMIP_degradation_2mins_eppie_Fig8B.csv",sep="") # get d
ata
df_A <- read.csv(FigA, sep = ',')
A <- ExtractRowIntoArray(df_A, 300)
dataList <- append(dataList, list(array2 = A))

FigA <- paste(wd, "T0380_12_Cos7H250_40nMIP_degradation_6mins_eppie_Fig8C.csv",sep="") # get d
ata
df_A <- read.csv(FigA, sep = ',')
A <- ExtractRowIntoArray(df_A, 300)
dataList <- append(dataList, list(array3 = A))

S <- StatsKruskal(dataList)
```

```
print(S$kruskal)
```

```
##
## Kruskal-Wallis rank sum test
##
## data: list_of_arrays
## Kruskal-Wallis chi-squared = 1555.9, df = 2, p-value < 2.2e-16</pre>
```

```
print(S$P)
```

```
## [1] "=====>>>>>> significance = *****
```

# Panel 8E vs 8F vs control: 7A: Norepinephrin with 0 s (control), 2 min or 6 min of LED illumination:

Absolute lifetimes at t = 300 are compared, noting that between these experiments, stimulation was identical timepoints. Teststatistic: lifetime value.

```
FigA <- paste(wd,"11_Cos7H250_200nMNE_in_FB_only_Fig7A.csv",sep="") # get data

df_A <- read.csv(FigA, sep = ',')

A <- ExtractRowIntoArray(df_A, 300)

dataList <- list(array1 = A)

FigA <- paste(wd,"T0455_13_Cos7H250_200nMNE_degradation_2mins_eppie_Fig8E.csv",sep="") # get

data

df_A <- read.csv(FigA, sep = ',')

A <- ExtractRowIntoArray(df_A, 300)

dataList <- append(dataList, list(array2 = A))

FigA <- paste(wd,"T0405_13_Cos7H250_200nMNE_degradation_6mins_eppie_Fig8F.csv",sep="") # get

data

df_A <- read.csv(FigA, sep = ',')

A <- ExtractRowIntoArray(df_A, 300)

dataList <- append(dataList, list(array3 = A))

S <- StatsKruskal(dataList)
```

```
print(S$kruskal)
```

```
##
## Kruskal-Wallis rank sum test
##
## data: list_of_arrays
## Kruskal-Wallis chi-squared = 1409.4, df = 2, p-value < 2.2e-16</pre>
```

```
print(S$P)
```

```
## [1] "====>>>>>> significance = ***** <<<<<====="
```

# Panel 7C vs 8G vs 8H: Adrenalin with 0 s (control), 2 min or 6 min of LED illumination:

Absolute lifetimes at t = 300 are compared, noting that between these experiments, stimulation was at identical timepoints. Teststatistic: lifetime value.

```
FigA <- paste(wd, "11_Cos7H250_250nMAdre_in_FB_only_Fig7C.csv", sep="") # get data
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df_A, 300)
dataList <- list(array1 = A)</pre>
FigA <- paste(wd, "T0340_13_Cos7H250_250nMAdre_degradation_2mins_eppie_Fig8G.csv", sep="") # ge
t data
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df A, 300)
dataList <- append(dataList, list(array2 = A))</pre>
FigA <- paste(wd, "T0425_13_Cos7H250_250nMAdre_degradation_6mins_eppie_Fig8H.csv", sep="") # ge
t data
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df_A, 300)
dataList <- append(dataList, list(array3 = A))</pre>
S <- StatsKruskal(dataList)</pre>
## Mean 3.279348
                              Var 0.0005154811
```

```
print(S$kruskal)
```

```
##
## Kruskal-Wallis rank sum test
##
## data: list_of_arrays
## Kruskal-Wallis chi-squared = 1141.3, df = 2, p-value < 2.2e-16</pre>
```

```
print(S$P)
```

```
## [1] "=====>>>>>> significance = *****
```

## Panel 7E vs 8I vs 8J: Prostaglandin with 0 s (control), 2 min or 6 min of LED illumination:

Absolute lifetimes at timepoint of maximum stimulation are compared. Teststatistic: lifetime value.

```
FigA <- paste(wd,"11_Cos7H250_200nMPGE-1_in_FB_only_Fig7E.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df_A, 180)
dataList <- list(array1 = A)</pre>
FigA <- paste(wd, "T0406_13_Cos7H250_200nMPGE-1_degradation_2mins_eppie_Fig8I.csv", sep="") # g
et data
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df_A, 180)
dataList <- append(dataList, list(array2 = A))</pre>
FigA <- paste(wd, "T0406_13_Cos7H250_200nMPGE-1_degradation_6mins_eppie_Fig8J.csv", sep="") # g
et data
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df_A, 200)
dataList <- append(dataList, list(array3 = A))</pre>
S <- StatsKruskal(dataList)</pre>
## Mean 3.086965
                             Var 0.008278756
## Mean 3.082212
                             Var 0.01244308
## Mean 3.089186
                             Var 0.006797883
print(S$kruskal)
```

```
##
## Kruskal-Wallis rank sum test
##
## data: list_of_arrays
## Kruskal-Wallis chi-squared = 0.021175, df = 2, p-value = 0.9895
```

```
print(S$P)
```

```
## [1] "=====>>>>>> data do not differ significantly <<<<<====="
```

### Figure 10. Effects of Folic acid.

# Panel A vs B vs C: Isoproterenol stimulation with 0 s (control), 2 min and 6 min of exposure to LED light:

Responses after stimulation, just before addition of forskolin are compared. Teststatistic: lifetime value.

```
FigA <- paste(wd,"14_Cos7H250_40nMIP_degradation_0mins_eppie_HBS++_FA_Fig10A.csv",sep="") # g
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df A, 400)
dataList <- list(array1 = A)</pre>
FigA <- paste(wd, "T0425_14_Cos7H250_40nMIP_degradation_2mins_eppie_HBS++_FA_Fig10B.csv", sep
="") # get data
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df_A, 400)
dataList <- append(dataList, list(array2 = A))</pre>
FigA <- paste(wd, "T0531_14_Cos7H250_40nMIP_degradation_6mins_eppie_HBS++_FA_Fig10C.csv", sep
="") # get data
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df_A, 400)
dataList <- append(dataList, list(array3 = A))</pre>
S <- StatsKruskal(dataList)</pre>
## Mean 3.279881
                             Var 0.0004879474
                             Var 0.014296
## Mean 3.114069
```

```
## Mean 2.71332
                          Var 0.007782577
```

```
print(S$kruskal)
```

```
##
##
   Kruskal-Wallis rank sum test
## data: list_of_arrays
## Kruskal-Wallis chi-squared = 1524.1, df = 2, p-value < 2.2e-16
```

```
print(S$P)
```

```
## [1] "====>>>>>>
                        significance = *****
                                                <<<<<<======"
```

### Panel E vs F vs G: Isoproterenol in saline with folic acid w/wo LED illumination, and rescue by inclusion of ascorbic acid:

Responses after stimulation and upon subsequent exposure to LED light are compared. Teststatistic: lifetime value.

```
FigA <- paste(wd, "15_Cos7H250_40nMIP_sustained_LED_2mins_HBS++_only_cut_to_997s_Fig10E.csv", s
ep="") # get data
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df_A, 750)
dataList <- list(array1 = A)</pre>
FigA <- paste(wd, "15_Cos7H250_40nMIP_LED_2mins_HBS++_FA_option3_Fig10F.csv", sep="") # get dat
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df_A, 750)
dataList <- append(dataList, list(array2 = A))</pre>
FigA <- paste(wd,"15_Cos7H250_40nMIP_sustained_LED_2mins_HBS++_FA_AA_RESCUE_cut_to_997s_Fig10
G.csv",sep="") # get data
df_A <- read.csv(FigA, sep = ',')</pre>
A <- ExtractRowIntoArray(df_A, 750)
dataList <- append(dataList, list(array3 = A))</pre>
S <- StatsKruskal(dataList)</pre>
## Mean 3.267984
                             Var 0.001180006
```

```
print(S$kruskal)
```

```
##
## Kruskal-Wallis rank sum test
##
## data: list_of_arrays
## Kruskal-Wallis chi-squared = 1402.8, df = 2, p-value < 2.2e-16</pre>
```

```
print(S$P)
```

```
## [1] "=====>>>>>> significance = ***** <<<<<====="
```