Automatic generation of paper figures

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1 Introduction

In this vignette we automatically generate the data analysis figures produced for the paper by Guglielmi *et al*.

2 Load Data

```
data("opto")
library(dplyr)
library(ggplot2)
```

3 Analysis

3.1 WT

To identify the furrowing line in the control (WT) samples, we first chose by inspection a time point for each sample that showed a clear band of constriction. An average cell area profile was computed along the dorsal-ventral (d-v) axis, to which a smoothing line was fitted. We defined the position of minimum area as the furrowing position. Here we compute this for all of the control samples but display the results for only one sample.

```
tabWT <- filter(opto, condition == "WT")
myCex <- 1.4
tabWT[tabWT$sample == "Global Activation/1_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOffset5e-04_closingSize5
tabWT[tabWT$sample == "Global Activation/2_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOffset5e-04_closingSize5
tabWT[tabWT$sample == "Global Activation/3_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOffset5e-04_closingSize5
tabWT[tabWT$sample == "Global Activation/4_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOffset5e-04_closingSize5
tabWT[tabWT$sample == "Global Activation/5_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOffset5e-04_closingSize5

plotSamples <- "Global Activation/4_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOffset5e-04_closingSize5.rda"
fnPos <- function(x, nbinsExclude=150, myCex=1.4, plotSamples) {
    doPlot <- x$sample[1] %in% plotSamples
    pos <- identifyFurrowPosition(x, nbinsExclude=nbinsExclude, h=100,
        plot=doPlot, myCex=myCex, px=x$px[1])
    return(data.frame(furrowPos=pos))
}
dvFurrowPos <- tabWT %>%
    group_by(sample) %>%
    filter(t == tstar) %>%
    do(fnPos(., plotSamples=""))
```

```
## Warning:  'as.tbl()' is deprecated as of dplyr 1.0.0.
## Please use 'tibble::as_tibble()' instead.
## This warning is displayed once every 8 hours.
## Call 'lifecycle::last_warnings()' to see where this warning was generated.
```

```
for (i in seq_along(dvFurrowPos$sample))
    tabWT[tabWT$sample %in% dvFurrowPos$sample[i], "furrowPos"] <-
        dvFurrowPos$furrowPos[i]
print(dvFurrowPos)
```

```
## # A tibble: 5 x 2
## # Groups:   sample [5]
##   sample                                                      furrowPos
##   <chr>                                                           <int>
## 1 Global Activation/1_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOff~       301
## 2 Global Activation/2_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOff~       258
## 3 Global Activation/3_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOff~       280
## 4 Global Activation/4_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOff~       207
## 5 Global Activation/5_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOff~       266
```

We wished to focus only on cells along or near to the furrowing line. Therefore we subsetted the control data to include only cells located in a box of dimensions 30 x 10 microns that was centered on the furrowing line.

```
L <- function(x, px) round(microns2px(x, px))
fnBox <- function(x, w=512, Lx=30, Ly=10) {
    Lx <- L(Lx, px=x$px[1])
    Ly <- L(Ly, px=x$px[1])
    box <- constructBox(x$furrowPos[1], Lx=Lx, Ly=Ly, w=w)
    return(data.frame(t(box)))
}
boxes <- tabWT %>% group_by(sample) %>% do(fnBox(.))

fnIsolate <- function(x, boxes) {
    box <- unlist(filter(boxes, sample == x$sample[1])[2:5])
    x <- isolateBoxCells(x, box)
    return(x)
}
tabWTs <- tabWT %>% group_by(sample) %>% do(fnIsolate(., boxes=boxes))
```

We then proceeded to also automatically identify the time point of tissue invagination. We noticed that as the tissue furrowed, adjacent cells moved into the observation box from both dorsal and ventral sides, and tended to be larger in area than the cells that initiated the constriction. By fitting a smoothing line to the time profile of median cell area in the observation box it was straightforward to identify the time at which a minimum area was reached, which we chose to define as the time point of tissue invagination.

```
fnMinArea <- function(x, plot=FALSE) {
    px <- x$px[1]
    res <- identifyTimeMinArea(x=x, px=px, plot=plot)
    return(data.frame(t=res["tindex"]))
}
tMinArea <- tabWTs %>% group_by(sample) %>% do(fnMinArea(.))
for (i in seq_along(tMinArea$sample))
    tabWTs[tabWTs$sample == tMinArea$sample[i], "tstar"] <- tMinArea$t[i]
tabWTs <- filter(tabWTs, t <= tstar)
```

Next we inspected the dynamics of cell area and anisotropy.

```
plotSample <- "Global Activation/4_CTRL_VFF.lsm - C=0/furrowSeg1.0.47_threshOffset5e-04_closingSize5.rda"
tabSample <- filter(tabWTs, sample == plotSample)
tabSample$t <- tabSample$t*tabSample$dt
plotFeatureEvolution(tabSample, dt=1/60, tMax=Inf, myTitle="wild-type",
    px=tabSample$px[1], cex=2, cex.axis=1.5, line=3.3,
    mar=c(5.1, 5.6, 4.1, 4.6))
```

A summary of WT cell features at the time point of furrowing was then taken.

```
summaryWT <- tabWTs %>%
    group_by(sample) %>%
    filter(t == tstar) %>%
    summarize(APanisotropy=median(e.x, na.rm=TRUE),
        APanisotropy25=quantile(e.x, probs=0.25, na.rm=TRUE),
```

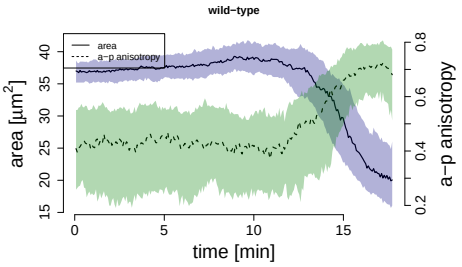

Figure 1: Fig 2J: Cell area and a-p anisotropy are plotted over time for cells within an area of 30 microns x 10 microns along the furrowing line for one control sample
Solid and dashed lines indicate the median over cells for area and a-p anisotropy, respectively, while shaded regions show the interquartile range.

```
        APanisotropy75=quantile(e.x, probs=0.75, na.rm=TRUE),
        area=median(x.0.s.area, na.rm=TRUE),
        area25=quantile(x.0.s.area, probs=0.25, na.rm=TRUE),
        area75=quantile(x.0.s.area, probs=0.75, na.rm=TRUE))

## `summarise()` ungrouping output (override with `.groups` argument)

summaryWTapicalSlice <- tabWTs  %>%
    group_by(sample) %>%
    filter(t == tstar, z == 1) %>%
    summarize(APanisotropy=median(e.x, na.rm=TRUE),
        APanisotropy25=quantile(e.x, probs=0.25, na.rm=TRUE),
        APanisotropy75=quantile(e.x, probs=0.75, na.rm=TRUE),
        area=median(x.0.s.area, na.rm=TRUE),
        area25=quantile(x.0.s.area, probs=0.25, na.rm=TRUE),
        area75=quantile(x.0.s.area, probs=0.75, na.rm=TRUE))

## `summarise()` ungrouping output (override with `.groups` argument)
```

3.2 Global

We first inspected the dynamics of samples that were globally activated.

```
tabGlobal <- filter(opto, condition == "Global")
tabGlobal$t <- tabGlobal$t*tabGlobal$dt
plotSample <- "Global Activation/Global Activation/Image31.lsm - C=0/furrowSeg1.0.47_threshOffset5e-04_closi
tabSample <- filter(tabGlobal, sample == plotSample)
plotFeatureEvolution(tabSample, dt=1/60, tMax=30, myTitle="globally activated",
    px=tabSample$px[1], cex=2, cex.axis=1.5, line=3.3,
    mar=c(5.1, 5.6, 4.1, 4.6))
```

We then asked if the cell behaviour of the control and photo-activated samples were quantitatively different. Since there was no clear way to choose equivalent time points in the control and photo-activated samples, we decided to compare control cells at the time of invagination to photo-activated cells at 10, 20 and 30 minutes post-activation.

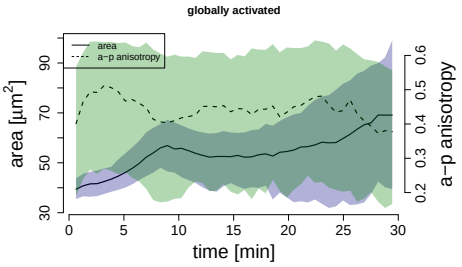

Figure 2: **Fig 2K: Cell area and a-p anisotropy are plotted over time for all cells in a globally light-activated sample**
Solid and dashed lines indicate the median over cells for area and a-p anisotropy, respectively, while shaded regions show the interquartile range.

```
sampleCenter <- tabGlobal %>% group_by(sample) %>% summarize(pos=mean(x.0.m.cy))

## `summarise()` ungrouping output (override with `.groups` argument)

for (i in seq_len(nrow(sampleCenter)))
    tabGlobal[tabGlobal$sample == sampleCenter$sample[i], "furrowPos"] <-
        sampleCenter$pos[i]
subsetTimes <- function(x, tstar) {
    times <- unique(x$t)
    ind <- which.min(abs(tstar-times/60))
    xs <- filter(x, t == times[ind])
    return(xs)
}
compareTimes <- c(10, 20, 30)
tabGlobals <- lapply(compareTimes, function(tstar) {
    y <- tabGlobal %>% group_by(sample) %>% do(subsetTimes(., tstar=tstar))
    y$tstar <- tstar
    return(y)
})
tabGlobals <- bind_rows(tabGlobals)
tabGlobals$condition <- paste0("Global", tabGlobals$tstar)
compareGlobal <- tabGlobals %>% group_by(condition, sample) %>%
    summarize(APanisotropy=median(e.x, na.rm=TRUE),
        APanisotropy25=quantile(e.x, probs=0.25, na.rm=TRUE),
        APanisotropy75=quantile(e.x, probs=0.75, na.rm=TRUE),
        area=median(x.0.s.area, na.rm=TRUE),
        area25=quantile(x.0.s.area, probs=0.25, na.rm=TRUE),
        area75=quantile(x.0.s.area, probs=0.75, na.rm=TRUE))

## `summarise()` regrouping output by 'condition' (override with `.groups` argument)

summaryWT$condition <- "control"
compareGlobal <- bind_rows(compareGlobal, summaryWT)
```

To assess differences we then performed two-sample t-tests on the median cell area and a-p anisotropy for each sample. Since we compared between multiple groups, multiple testing correction was performed using the method of Bonferroni.

```
wtArea <- compareGlobal %>% filter(condition == "control") %>%
    summarize(meanArea=mean(area))

## `summarise()` ungrouping output (override with `.groups` argument)

wtArea <- wtArea$meanArea
compareGlobal$normArea <- log2(compareGlobal$area/wtArea)
compareGlobal$normArea25 <- log2(compareGlobal$area25/wtArea)
compareGlobal$normArea75 <- log2(compareGlobal$area75/wtArea)
ttAPanisotropy <- pairwise.t.test(compareGlobal$APanisotropy,
    compareGlobal$condition, p.adjust.method="bonferroni")
print(ttAPanisotropy)

##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  compareGlobal$APanisotropy and compareGlobal$condition
##
##          Global10 Global20 Global30
## Global20 1.00000  -        -
## Global30 1.00000  1.00000  -
## control  0.00016  0.00075  0.00166
##
## P value adjustment method: bonferroni

ttArea <- pairwise.t.test(compareGlobal$normArea, compareGlobal$condition,
    p.adjust.method="bonferroni")
print(ttArea)

##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  compareGlobal$normArea and compareGlobal$condition
##
##          Global10 Global20 Global30
## Global20 1        -        -
## Global30 1        1        -
## control  9.3e-07  8.8e-07  1.2e-07
##
## P value adjustment method: bonferroni
```

For visualization purposes, p-values were converted to stars indicating significance levels. For the comparison of median cell areas, the testing was performed on log2 transformed values to ensure a more constant variance between the conditions. To ensure that we were log transforming a dimensionless quantity, we first divided each measurement by the mean of the areas in the control group. We also decided to show the corresponding absolute area measurements on the right-hand axis.

```
mySignIf <- function(pv) symnum(pv, cutpoints=c(0, 0.001, 0.01, 0.05, 1),
    symbols=c("***", "**", "*", "n.s."))
myCol <- c("red", rep("blue", 3))
compareGlobal$condition <- factor(compareGlobal$condition,
    levels=c("control", "Global10", "Global20", "Global30"))
myNames <- levels(compareGlobal$condition)
```

```
myStarsAPanisotropy <- apply(ttAPanisotropy$p.value, c(1, 2), mySignIf)
info <- sessionInfo()
par(mar=c(7.1, 6.1, 3.1, 5.6))
stripchart(APanisotropy ~ condition, data=compareGlobal, vertical=TRUE, pch=20,
    xlab="", ylab="a-p anisotropy", cex.lab=2, ylim=c(0, 1.1), col=myCol,
    axes=FALSE, method="jitter", jitter=0.05, cex=2)
axis(side=1, at=1:4, labels=FALSE)
text(x=1, par("usr")[3]-0.17, labels="control", srt=45, xpd=TRUE, cex=2)
text(x=2:4, par("usr")[3]-0.12, labels=rep("light", 3), srt=45, xpd=TRUE, cex=2)
text(x=2:4+0.05, par("usr")[3]-0.2, labels=c("+10min", "+20min", "+30min"),
     srt=45, pos=NULL, xpd=TRUE, cex=2)
axis(side=2, cex.axis=1.5, lwd=1.5)
drawBar <- function(x0, x1, y, star, offset=0.1, cex=2) {
    segments(x0=x0, y0=y, x1=x1, y1=y)
    text(x=mean(c(x0, x1)), y=y+offset, labels=star, cex=cex)
}
drawBar(1, 2, y=0.85, star=myStarsAPanisotropy["control", "Global10"],
    offset=0.05)
drawBar(1, 3, y=0.95, star=myStarsAPanisotropy["control", "Global20"],
    offset=0.05)
drawBar(1, 4, y=1.05, star=myStarsAPanisotropy["control", "Global30"],
    offset=0.05)
drawBar(2, 3, y=0.23, star=myStarsAPanisotropy["Global20", "Global10"],
    offset=-0.05)
drawBar(2, 4, y=0.65, star=myStarsAPanisotropy["Global30", "Global10"],
    offset=0.05)
drawBar(3, 4, y=0.13, star=myStarsAPanisotropy["Global30", "Global20"],
    offset=-0.05)
conditionSummary <- compareGlobal %>% group_by(condition) %>%
    summarize(median=median(APanisotropy),
        q25=quantile(APanisotropy, probs=0.25),
        q75=quantile(APanisotropy, probs=0.75))

## `summarise()` ungrouping output (override with `.groups` argument)

conditionSummary <- as.data.frame(conditionSummary)
conditionSummary <- conditionSummary[order(conditionSummary$condition), ]
for (i in seq_len(nrow(conditionSummary))) {
    segments(x0=i-0.2, x1=i+0.2, y0=conditionSummary[i, "median"], lwd=2, lty=1)
    segments(x0=i, y0=conditionSummary[i, "q25"], y1=conditionSummary[i, "q75"],
        lwd=2, lty=1)
}
myStarsArea <- apply(ttArea$p.value, c(1, 2), mySignIf)
par(mar=c(7.1, 6.1, 3.1, 5.6))
stripchart(normArea ~ condition, data=compareGlobal, vertical=TRUE, pch=20,
    xlab="", ylab=expression(paste("log"[2], "(area / <area>"[ctrl], ")")),
    cex.lab=2, group.names=myNames, ylim=c(-0.5, 2.0), col=myCol,
    axes=FALSE, method="jitter", jitter=0.05, cex=2)
axis(side=1, at=1:4, labels=FALSE)
text(x=1, par("usr")[3]-0.47, labels="control", srt=45, xpd=TRUE, cex=2)
text(x=2:4, par("usr")[3]-0.32, labels=rep("light", 3), srt=45, xpd=TRUE, cex=2)
text(x=2:4+0.05, par("usr")[3]-0.53, labels=c("+10min", "+20min", "+30min"),
```

```
     srt=45, pos=NULL, xpd=TRUE, cex=2)
axis(side=2, cex.axis=1.5, lwd=1.5)
drawBar(1, 2, y=1.3, star=myStarsArea["control", "Global10"], offset=0.12)
drawBar(1, 3, y=1.6, star=myStarsArea["control", "Global20"], offset=0.12)
drawBar(1, 4, y=1.9, star=myStarsArea["control", "Global30"], offset=0.12)
drawBar(2, 3, y=0.40, star=myStarsArea["Global20", "Global10"], offset=-0.12)
drawBar(2, 4, y=0.20, star=myStarsArea["Global30", "Global10"], offset=-0.12)
drawBar(3, 4, y=0.60, star=myStarsArea["Global30", "Global20"], offset=-0.12)
mapMicronsSq <- function(x) px2area(wtArea*2**x, px=0.293)
rangeNormAreaSeq <- seq(-0.5, 2.0, by=0.5)
newAxisLabels <- round(mapMicronsSq(rangeNormAreaSeq), digits=1)
axis(4, at=rangeNormAreaSeq, labels=newAxisLabels, cex.axis=1.5, lwd=1.5)
abline(h=0, lty=2, col="blue")
mtext(expression(paste("area [", mu, m^2, "]")), side=4, cex=2, line=4)
conditionSummary <- compareGlobal %>% group_by(condition) %>%
    summarize(median=median(normArea), q25=quantile(normArea, probs=0.25),
        q75=quantile(normArea, probs=0.75))

## `summarise()` ungrouping output (override with `.groups` argument)

conditionSummary <- as.data.frame(conditionSummary)
conditionSummary <- conditionSummary[order(conditionSummary$condition), ]
for (i in seq_len(nrow(conditionSummary))) {
    segments(x0=i-0.2, x1=i+0.2, y0=conditionSummary[i, "median"], lwd=2, lty=1)
    segments(x0=i, y0=conditionSummary[i, "q25"], y1=conditionSummary[i, "q75"],
        lwd=2, lty=1)
}
```

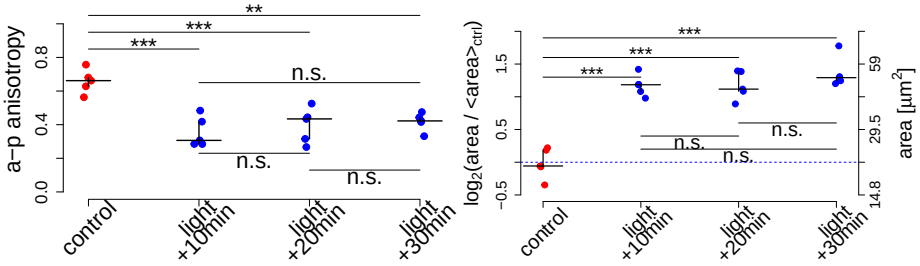

Figure 3: Fig 2L-M: The median cell area and a-p anisotropy features at the time point of tissue invagination in the control samples were compared to three different time points in the photo-activated samples

The statistical testing was performed using pairwise two-sample t-tests. As can be seen from the figure, standard deviations were comparable between groups. To attain a higher precision we therefore used a pooled standard deviation for all of the tests. p-values were corrected for multiple testing using the method of Bonferroni. We performed the area testing on the log-transformed values but included the absolute areas on the right-hand y-axis as a reference. For both cell area and a-p anisotropy, the control samples were significantly different from the photo-activated samples, while the the latter showed no significant changes over time. The crosses show group median (horizontal line) and interquartile range (vertical line).

3.3 2p titration

To assess the effect of increasing laser power on cell behaviour we extracted cell features from the following samples.

```
titrationCond <- paste0("2pTitration", c("2.5", "5", "8"), "percent")
tab2pTitration <- filter(opto, condition %in% titrationCond)
tab2pTitration <- filter(tab2pTitration, !(sample == "New data/2p titration/2.5%/1_CIBNOCRL__R001_GR1_B1_SUM/
tab2pTitration <- filter(tab2pTitration, !(sample == "New data/2p titration/2.5%/2_CIBNOCRL__R001_GR1_B1_SUM/
tab2pTitration <- filter(tab2pTitration, !(sample == "New data/2p titration/2.5%/3_CIBNOCRL__R001_GR1_B1_SUM/
tab2pTitration$t <- tab2pTitration$t*tab2pTitration$dt
plotSamples <- c(
    "700 uW"="New data/2p titration/2.5%/2_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04_closi
    "1.5 mW"="New data/2p titration/5%/1_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04_closingS
    "3 mW"="New data/2p titration/8%/4_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04_closingSiz
)
for (i in seq_along(plotSamples)) {
    s <- plotSamples[i]
    tab2pTitrations <- filter(tab2pTitration, sample == s)
    px <- tab2pTitrations$px[1]
    plotFeatureEvolution(tab2pTitrations, dt=1/60, tMax=Inf, myTitle=names(s),
        px=px, cex=2, cex.axis=1.5, line=3.3, mar=c(5.1, 5.6, 4.1, 4.6))
}
```

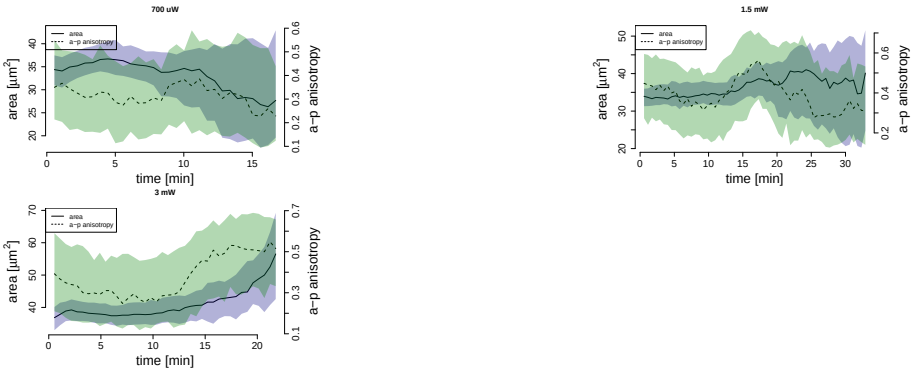

Figure 4: **Fig 5M-O: Cell area and a-p anisotropy are plotted over time for locally light-activated cells in individual samples**
Different two-photon laser powers were used to monitor changes in cell behaviour resulting from varying levels of activation. Solid and dashed lines indicate the median over cells for area and a-p anisotropy, respectively, while shaded regions show the interquartile range.

3.4 Sktl, Zip, Dark

Next we tested if a-p anisotropy from various control samples was different from wild-type samples at the time point of furrowing. The most apical z-stack that gave an accurate cell segmentation was kept for the following analysis. A box isolating a group of constricting cells was chosen manually by the biologist.

```
tab <- filter(opto, condition %in% c("Dark", "Sktl", "Zip"))
summaryConds <- tab %>% group_by(condition, sample) %>%
    summarize(APanisotropy=median(e.x, na.rm=TRUE),
        APanisotropy25=quantile(e.x, probs=0.25, na.rm=TRUE),
        APanisotropy75=quantile(e.x, probs=0.75, na.rm=TRUE))
```

```
## `summarise()` regrouping output by 'condition' (override with `.groups` argument)

summaryWTapicalSlice$condition <- "WT"
summaryConds <- bind_rows(summaryConds, summaryWTapicalSlice)
myCol <- c(rep("blue", 3), "red")
summaryConds$condition <- factor(summaryConds$condition,
    levels=c("Dark", "Sktl", "Zip", "WT"))
summaryConds$sample <- factor(summaryConds$sample)
sampleOrdering <- levels(summaryConds$sample)[c(6:15, 1:5)]
summaryConds$sample <- factor(summaryConds$sample, levels=sampleOrdering)
anisotropyLab <- "a-p anisotropy"
par(mar=c(6.1, 6.1, 3.1, 2.1))
stripchart(APanisotropy ~ condition, data=summaryConds, vertical=TRUE, pch=20,
    xlab="", ylab=anisotropyLab, cex.lab=2, axes=FALSE, col=myCol,
    ylim=c(0, 1.1), method="jitter", jitter=0.05, cex=2)
axis(side=1, at=1:4, labels=FALSE, cex.axis=1.5, lwd=1.5)
axis(side=2, cex.axis=1.5, lwd=1.5)
text(x=1:4, par("usr")[3]-0.12, labels=levels(summaryConds$condition), srt=45,
    xpd=TRUE, cex=2)
conditionSummary <- summaryConds %>% group_by(condition) %>%
    summarize(median=median(APanisotropy),
        q25=quantile(APanisotropy, probs=0.25),
        q75=quantile(APanisotropy, probs=0.75))

## `summarise()` ungrouping output (override with `.groups` argument)

conditionSummary <- as.data.frame(conditionSummary)
for (i in seq_len(nrow(conditionSummary))) {
    segments(x0=i-0.2, x1=i+0.2, y0=conditionSummary[i, "median"], lwd=2, lty=1)
    segments(x0=i, y0=conditionSummary[i, "q25"], y1=conditionSummary[i, "q75"],
        lwd=2, lty=1)
}
ttConds <- pairwise.t.test(summaryConds$APanisotropy, summaryConds$condition,
    p.adjust.method="bonferroni")
myStarsConds <- apply(ttConds$p.value, c(1, 2), mySignIf)
print(myStarsConds)

##      Dark   Sktl   Zip
## Sktl "n.s." "?"    "?"
## Zip  "n.s." "n.s." "?"
## WT   "n.s." "n.s." "n.s."

drawBar(1, 2, y=0.40, star=myStarsConds["Sktl", "Dark"], offset=-0.05)
drawBar(2, 3, y=0.30, star=myStarsConds["Zip", "Sktl"], offset=-0.05)
drawBar(1, 3, y=0.20, star=myStarsConds["Zip", "Dark"], offset=-0.05)
drawBar(1, 4, y=0.10, star=myStarsConds["WT", "Dark"], offset=-0.05)
drawBar(2, 4, y=0.90, star=myStarsConds["WT", "Sktl"], offset=0.05)
drawBar(3, 4, y=0.80, star=myStarsConds["WT", "Zip"], offset=0.05)
```

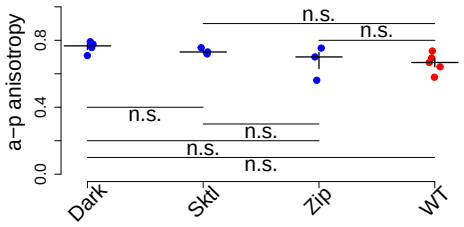

Figure 5: **Fig 3A: The median a-p anisotropy of constricting cells in different condition are compared to the a-p anisotropy of control cells at the time point of furrowing**
The statistical testing was performed using pairwise two-sample t-tests. As can be seen from the figure, standard deviations were comparable between groups. To attain a higher precision we therefore used a pooled standard deviation for all of the tests. p-values were corrected for multiple testing using the method of Bonferroni. No significant differences were detected between the groups. The crosses show group median (horizontal line) and interquartile range (vertical line).

3.5 Boxes Far vs Boxes Close

We wished to compare samples from the double activation experiments where two boxes of activation were placed at different distances from one another. Specifically, we wished to quantify the a-p anisotropy of cells in the central area that was not activated. As the mCherry signal in this region was predominantly cytoplasmic, it was not possible to obtain an accurate segmentation of cell shape. We therefore instead imaged a single time point at the end of each experiment in the GFP channel, with which cell shapes could be resolved. For this section the most apical z-stack that gave an accurate cell segmentation was kept for the analysis. A box isolating a group of constricting cells was chosen manually by the biologist.

```
tabBoxes <- filter(opto, condition %in% c("BoxesFar", "NewBoxesFar",
    "BoxesClose"))
summaryBoxes <- tabBoxes %>% group_by(condition, sample) %>%
    summarize(APanisotropy=median(e.x, na.rm=TRUE),
        APanisotropy25=quantile(e.x, probs=0.25, na.rm=TRUE),
        APanisotropy75=quantile(e.x, probs=0.75, na.rm=TRUE))
```

```
## `summarise()` regrouping output by 'condition' (override with `.groups` argument)
```

```
summaryBoxes$condition[summaryBoxes$condition == "NewBoxesFar"] <- "BoxesFar"
summaryBoxes$condition <- droplevels(summaryBoxes$condition)

ttDouble <- t.test(APanisotropy ~ condition, summaryBoxes)
print(ttDouble)
```

```
##
##  Welch Two Sample t-test
##
## data:  APanisotropy by condition
## t = -3.7062, df = 8.3088, p-value = 0.005603
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -0.35250238 -0.08317076
## sample estimates:
## mean in group BoxesClose   mean in group BoxesFar
##                0.4211512                0.6389877
```

```
diff(ttDouble$estimate)

## mean in group BoxesFar
##              0.2178366

par(mar=c(6.1, 6.1, 3.1, 2.1))
stripchart(APanisotropy ~ condition, summaryBoxes, vertical=TRUE, cex.lab=2,
    xlab="", ylab="a-p anisotropy", xlim=c(0.5, 2.5), ylim=c(0, 1), pch=19,
    col=c("blue", "red"), method="jitter", jitter=0.1, axes=FALSE, cex=2)
axis(side=1, at=1:2, labels=FALSE, lwd=1.5)
text(x=1:2, par("usr")[3]-0.06, labels=rep("Boxes", 2), srt=45, pos=1, xpd=TRUE,
    cex=2)
text(x=1:2+0.05, par("usr")[3]-0.13, labels=c("Close", "Far"), srt=45, pos=1,
    xpd=TRUE, cex=2)
axis(2, cex.axis=1.5, lwd=1.5)
drawBar(1, 2, y=0.9, star=mySignIf(ttDouble$p.value), offset=0.1, cex=2)
conditionSummary <- summaryBoxes %>% group_by(condition) %>%
    summarize(median=median(APanisotropy),
        q25=quantile(APanisotropy, probs=0.25),
        q75=quantile(APanisotropy, probs=0.75))

## `summarise()` ungrouping output (override with `.groups` argument)

conditionSummary <- as.data.frame(conditionSummary)
for (i in seq_len(nrow(conditionSummary))) {
    segments(x0=i-0.1, x1=i+0.1, y0=conditionSummary[i, "median"], lwd=2, lty=1)
    segments(x0=i, y0=conditionSummary[i, "q25"], y1=conditionSummary[i, "q75"],
        lwd=2, lty=1)
}
```

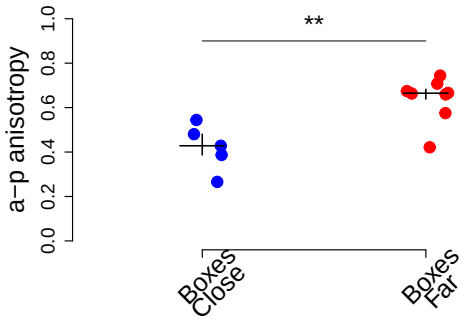

Figure 6: **Fig 7G: The median a-p anisotropy of groups of constricting cells were compared between samples where the distance between two boxes of light activation was varied**
A two-sample t-test revealed that cells were more elongated along the embryo a-p axis in the experiment where the boxes were placed farther apart. As can be seen from the plot, the two groups showed unequal variances. We therefore used Welch's t-test. The crosses show group median (horizontal line) and interquartile range (vertical line). The magnitude of difference in a-p anisotropy between the two groups was found to be 0.23.

3.6 Global 2p

We assessed the a-p anisotropy of samples that were globally activated with the 2-photon microscope at different powers.

```
tabPower700uW <- filter(opto, condition == "700uW")
tabPower700uW[tabPower700uW$sample == "New data/700uW/1_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffse
tabPower700uW[tabPower700uW$sample == "New data/700uW/2_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffse
tabPower700uW[tabPower700uW$sample == "New data/700uW/3_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffse
plotSamples700uW <- "New data/700uW/3_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04_closingSize
dvFurrowPos <- tabPower700uW %>% group_by(sample) %>% filter(t == tstar) %>%
    do(fnPos(., plotSamples="", nbinsExclude=0))
for (i in seq_along(dvFurrowPos$sample))
    tabPower700uW[tabPower700uW$sample %in% dvFurrowPos$sample[i], "furrowPos"] <- dvFurrowPos$furrowPos[i]
boxes <- tabPower700uW %>% group_by(sample) %>% do(fnBox(., w=724))
tabPower700uWs <- tabPower700uW %>% group_by(sample) %>%
    do(fnIsolate(., boxes=boxes))
tMinArea <- tabPower700uWs %>% group_by(sample) %>% do(fnMinArea(., plot=FALSE))
for (i in seq_along(tMinArea$sample))
    tabPower700uWs[tabPower700uWs$sample == tMinArea$sample[i], "tstar"] <- tMinArea$t[i]
tabPower700uWs <- filter(tabPower700uWs, t <= tstar)
```

```
plotSamples1.5mW <- "New data/Global_2p/2_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04_closing
tabPower1.5mW <- filter(opto, condition == "Global2p")
tabPower1.5mW$condition <- "1.5mW"
tabPower1.5mW[tabPower1.5mW$sample == "New data/Global_2p/2_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshO
tabPower1.5mW[tabPower1.5mW$sample == "New data/Global_2p/4_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshO
tabPower1.5mW[tabPower1.5mW$sample == "New data/Global_2p/8_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshO
dvFurrowPos <- tabPower1.5mW %>% group_by(sample) %>% filter(t == tstar) %>%
    do(fnPos(., plotSamples="", nbinsExclude=0))
for (i in seq_along(dvFurrowPos$sample))
    tabPower1.5mW[tabPower1.5mW$sample %in% dvFurrowPos$sample[i], "furrowPos"] <- dvFurrowPos$furrowPos[i]
boxes <- tabPower1.5mW %>% group_by(sample) %>% do(fnBox(., w=724))
tabPower1.5mWs <- tabPower1.5mW %>% group_by(sample) %>%
    do(fnIsolate(., boxes=boxes))
```

```
plotSamples3mW <- "New data/3mW/1_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04_closingSize3.rd
tabPower3mW <- filter(opto, condition == "3mW")
tabPower3mW[tabPower3mW$sample == "New data/3mW/1_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04
tabPower3mW[tabPower3mW$sample == "New data/3mW/2_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04
tabPower3mW[tabPower3mW$sample == "New data/3mW/3_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04
boxes <- tabPower3mW %>% group_by(sample) %>% do(fnBox(., w=724))
tabPower3mWs <- tabPower3mW %>% group_by(sample) %>% fnIsolate(., boxes=boxes)
```

```
tabPower <- bind_rows(tabPower700uWs, tabPower1.5mWs, tabPower3mWs)
tabPower$t <- tabPower$t*tabPower$dt
plotSamples <- c(
    "700 uW"="New data/700uW/3_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04_closingSize3.rda",
    "1.5 mW"="New data/Global_2p/2_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04_closingSize3.r
    "3 mW"="New data/3mW/1_CIBNOCRL__R001_GR1_B1_SUM/furrowSeg1.0.47_threshOffset5e-04_closingSize3.rda")
for (i in seq_along(plotSamples)) {
```

```
        s <- plotSamples[i]
        tabPowers <- filter(tabPower, sample == s)
        px <- tabPowers$px[1]
        plotFeatureEvolution(tabPowers, dt=1/60, tMax=Inf, myTitle=names(s),
            px=px, cex=2, cex.axis=1.5, line=3.3, mar=c(5.1, 5.6, 4.1, 4.6))
    }
```

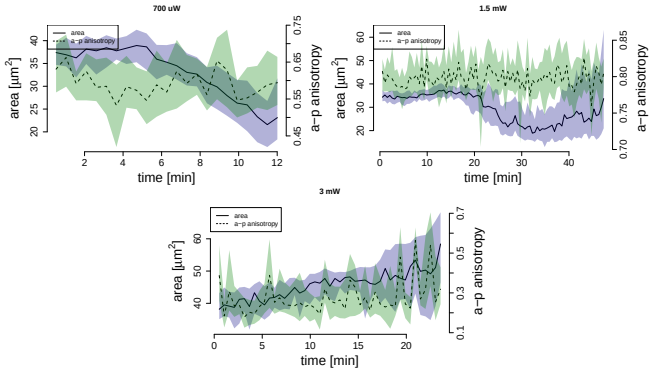

Figure 7: **Fig S4N-P: Cell area and a-p anisotropy are plotted over time for all cells in a globally light-activated sample that was activated with different two-photon laser powers**
Solid and dashed lines indicate the median over cells for area and a-p anisotropy, respectively, while shaded regions show the interquartile range.