

The statistical effects of measuring myocytes with different image zoom rates

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Abstract: Quantitative image measurements are widely used in both basic and clinical medical research. The measurements can be taken under different image zoom rates. Usually, increasing the image zoom rate increases the accuracy of the measurement; however, increasing the image zoom rate also increases the measurement cost. We measured 218 rat myocytes (112 myocytes from treated rat heart; 106 from placebo rat heart) for image measurements of cross-sectional areas, under different image zoom rates, 75%, 100%, 150%, 200%, and 300%. The linear mixed-effects model, Student's *t*-test, and *F* test were used to investigate whether image measurements significantly change over different zoom rates, and to find the optimal zoom rate in terms of maximizing the test statistic while reducing measurement cost. We found that the means of the measured myocyte areas decreased with increased zoom rates for both the treated heart and the placebo heart. The means of measured areas at zoom rate 75% versus 100% were not significantly different for both the treated heart and the placebo heart. However, there was a significant decrease when the zoom rate increased from 100% to 150% for the treated heart and the placebo heart, and the amount of decrease of the means for the two types of hearts was different. When the zoom rate was increased from 150% to 200% and 300%, the mean of the measurements decreased. However, the amount of decrease for both the treated heart and the placebo heart was the same. When image zoom rate increases, measurement precision remains the same. The *t*-test statistics for comparing treated heart and placebo heart remain the same when zoom rate reaches 150% and above. Therefore, the image zoom rate at 150% is the optimal choice in terms of maximizing the test statistic and reducing the measurement cost.

Keywords: image zoom rate, myocyte, cross-sectional area measurement, measurement cost, wheat germ agglutinin (WGA) staining, linear mixed-effects model

Introduction

Quantitative image measurements most often take place in either basic or clinical medical research, such as measurement for myocyte cross-sectional area and for capillary or vessel cross-sectional area. The measurement instruments can usually be adjusted to different magnification rates, which are often called image zoom rates. Larger zoom rate implies that the measured areas are magnified more and the number of experimental units measured per screen becomes less. Generally speaking, increasing the image zoom rate increases the accuracy of the measurement. However, increasing the image zoom rate also increases the measurement cost dramatically in terms of time and effort. Hence, there is a need to obtain an optimal zoom rate in which statistical test power is maximized, measurement cost is reduced, and measurement accuracy and precision are maintained.

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In this study, two rat hearts were used: a stem cell-treated heart (treatment) and a untreated heart (placebo). A total of 218 myocytes were selected, of which 112 myocytes were from the treated heart and 106 were from the placebo heart. For each myocyte, we took the measurements of the myocyte area under different zoom rates. We examined the changes of measurement accuracy and precision with the increasing image zoom rates during image measurement, and derived the optimal image zoom rate for obtaining a high-power test statistic with low measurement cost.

This paper does not cover any treatment effects but focuses only on the measurement effects caused by different image zoom rates. Readers who are interested in the treatment effects may refer to Tang et al.¹ To simplify the notation, the stem cell-treated heart is hereafter called the treated heart, and the untreated heart is called the placebo heart.

Materials and methods

Data collection

The images were acquired digitally under microscope (20×) in a TIFF format with a default resolution of 72 pixels/inch. The raw data were collected from image measurements of rat myocyte cross-sectional areas, which were determined by planimetry after staining cell membranes with green fluorescein isothiocyanate (FITC)-conjugated wheat germ agglutinin (WGA). The selection of myocytes for measurement was based on a clearly defined sarcolemma board and with associated round nuclei, which were stained with 4',6-diamidino-2-phenylindole (DAPI) (blue). In total, 218 myocytes were selected based on these criteria, of which 112 myocytes were from the treated heart, and 106 were from the placebo heart.

Measurement of the myocyte cross-sectional area was conducted with ImageJ v1.39u software (NIH, Bethesda, MD). ImageJ is an open source package sponsored by the National Institutes of Health (NIH) (<http://rsb.info.nih.gov/ij/download.html>). Figure 1 provides an illustration of the features of the selected myocytes. The solid blue color shows nuclei stained with DAPI. The green color shows cell membranes stained with FITC-conjugated WGA. The red lines are traced with ImageJ for cell area measurements.

The protocol for measuring the myocyte area is as follows.

1. Open the image with ImageJ (75% zoom rate by default).
2. Set up the measurement scale at 3.082/μm.
3. For each of the selected 218 myocytes, repeat step 4 and step 5.

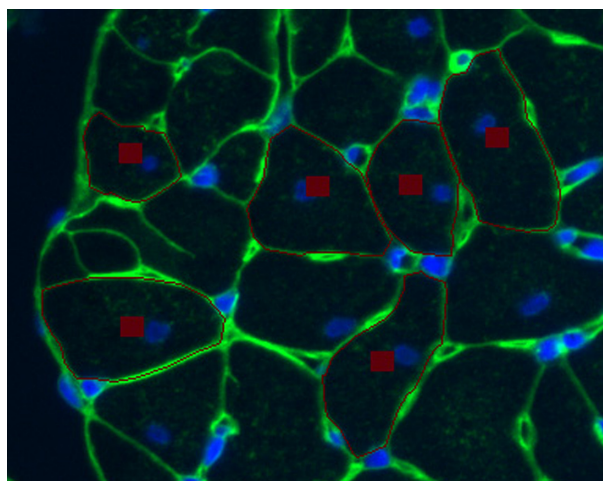


Figure 1 Illustration of myocyte selection for (see text for explanation of colors).

4. Trace the inner green edge of the myocyte
5. Measure the area
6. For other image zoom rates 100%, 150%, 200%, and 300%, repeat step 3 to step 5.

See Table 1 and Appendix II for the illustration of the collected data.

Statistical analysis

The mean and standard deviation for each type of heart at each zoom rate were calculated as shown in Table 2. We also report the *P*-values for testing the equality of the variances of the two types of hearts at each zoom rate, the pooled standard deviation, the mean difference, and the *t*-test statistic for testing the difference of the measured myocyte areas of the two types of hearts at each zoom rate. The linear mixed-effects model was used to examine whether and how the means of the measured myocyte areas changed over different image zoom rates.² The normality assumption for the linear mixed-effect model is valid based on the Q–Q plots of the measured myocyte areas for each type of heart and at each zoom rate. Student's *t*-tests (paired

Table 1 Illustration of the collected data

| Area ^a | Measurement type ^b | Group ^c | Myocyte ^d |
|-------------------|-------------------------------|--------------------|----------------------|
| 259.509 | 75 | Treated | 1 |
| 311.831 | 75 | Treated | 2 |
| 255.403 | 75 | Treated | 3 |
| ... | ... | ... | ... |
| 315.306 | 300 | Placebo | 104 |
| 377.946 | 300 | Placebo | 105 |
| 379.946 | 300 | Placebo | 106 |

Notes: ^aThe area of myocyte in the unit of μm², a dependent variable; ^bmeasurement type, representing different image measurement zoom rates, such as 75%, 100%, 150%, 200%, and 300%; ^cgroup factor, indicating whether the myocyte came from a treated heart or a placebo heart; ^dthe identification number for myocyte in each group, taking 1–112 for treatment and 1–106 for placebo.

Table 2 Basic descriptive statistics for the collected data

| | Zoom rate 75% | | Zoom rate 100% | | Zoom rate 150% | | Zoom rate 200% | | Zoom rate 300% | |
|------------------------------|---------------|---------|----------------|---------|----------------|---------|----------------|---------|----------------|---------|
| | Treatment | Placebo | Treatment | Placebo | Treatment | Placebo | Treatment | Placebo | Treatment | Placebo |
| N | 112 | 106 | 112 | 106 | 112 | 106 | 112 | 106 | 112 | 106 |
| Mean | 382.374 | 336.840 | 383.001 | 335.748 | 379.438 | 327.271 | 377.275 | 324.974 | 373.769 | 322.257 |
| Standard deviation | 103.682 | 101.187 | 103.944 | 101.688 | 103.507 | 101.383 | 102.783 | 100.951 | 102.044 | 100.244 |
| P-values for equal variances | 0.802 | | 0.822 | | 0.831 | | 0.854 | | 0.855 | |
| Pooled standard deviation | 102.476 | | 102.853 | | 102.480 | | 101.896 | | 101.173 | |
| Mean difference | 45.534 | | 47.253 | | 52.168 | | 52.301 | | 51.513 | |
| t-statistic | 3.279 | | 3.390 | | 3.757 | | 3.788 | | 3.757 | |

and unpaired) were used for analyses of measurement precision and inference on the optimal zoom rate.³ A significant level for α at 0.05 was adopted unless otherwise specified.

To examine whether the different zoom rates significantly change the measurements of the myocyte area, we first plotted the measurements of each myocyte area over different zoom rates (75%, 100%, 150%, 200%, and 300%) in Figure 2 as a thin line, where the solid thick line in each panel represents the means of the measured myocyte areas over different zoom rates for either the treated heart or the placebo heart. From Figure 2, it is clear that the between-myocyte variation is much larger than the within-myocyte variation. We used the linear mixed-effects model to examine the contribution of the different zoom rates on the measurements of a myocyte area. Let us denote y_i as the measured area for the i th myocyte at different zoom rates, ie, $y_i = (y_{i1}, y_{i2}, y_{i3}, y_{i4}, y_{i5})^T$. The linear mixed-effect model can be specified by the following:

$$y_{ij} = \beta_0 + \beta_1 I\{\text{treatment}\} + \beta_2 I\{\text{mt100}\} + \beta_3 I\{\text{mt150}\} + \beta_4 I\{\text{mt200}\} + \beta_5 I\{\text{mt300}\} + \beta_6 I\{\text{treatment} \& \text{mt100}\} + \beta_7 I\{\text{treatment} \& \text{mt150}\} + \beta_8 I\{\text{treatment} \& \text{mt200}\} + \beta_9 I\{\text{treatment} \& \text{mt300}\} + b_i + \varepsilon_{ij}. \quad (1)$$

Here, $I\{\}$ is an indicator function and takes a value of either 0 or 1. For example, if an observation is from the treated heart and is measured at a zoom rate of 100%, then $I\{\text{treatment}\}$, $I\{\text{mt100}\}$, and $I\{\text{treatment} \& \text{mt100}\}$ all take values of 1, and all other indicator functions take values of 0. With these notations, the expected values for the combination of different hearts and different zoom rates are presented in Table 3. The b_i in model (1) is assumed to be an identically and independently distributed random variable, with a normal distribution with mean 0 and variance σ_b^2 . The distribution of within-myocyte error ε_{ij} is usually assumed to be normally distributed with mean 0 and variance σ^2 . Generally, σ_b^2 describes the between-myocyte variation, and σ^2 describes the within-myocyte variation.

It is generally assumed that the observations for different myocytes are independent, and the within-myocyte observations are correlated. In model (1), the assumption that $b_i \sim N(0, \sigma_b^2)$ implies that the within-myocyte observations have the same level of correlations, which seems to be a reasonable assumption for this study. The parameters in model (1) can be obtained by using the lme function in the R package nlme,² which can be obtained from <http://www.r-project.org>.

Results

For model (1), the fitted results are presented in Table 4. From Table 4: (i) the estimates for β_2 , β_3 , β_4 , and β_5 are decreasing, which indicates that the means of the measurements of

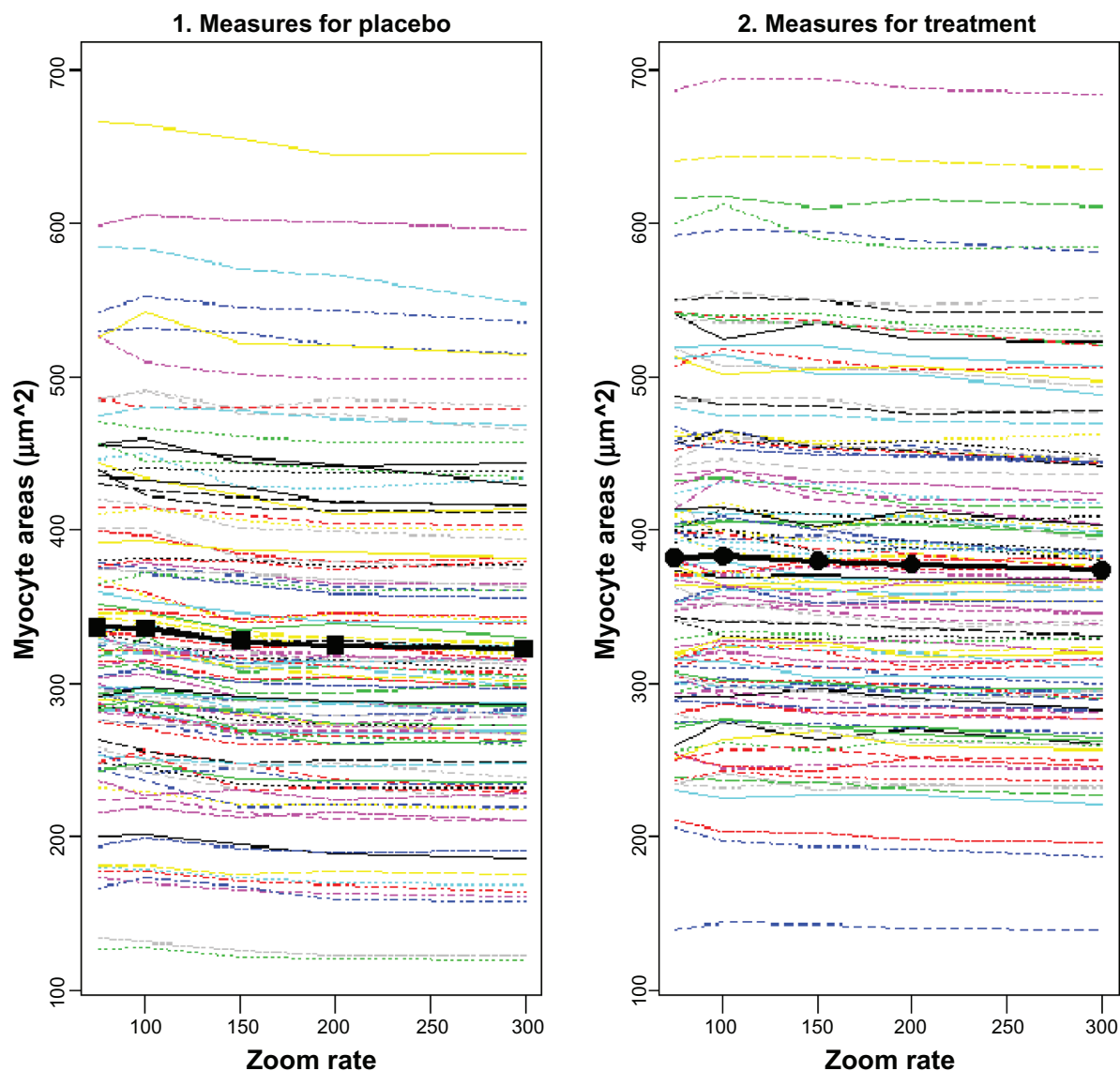


Figure 2 The measured myocyte areas for each myocyte over different zoom rates. The solid thick line in each panel connects the means of the myocyte areas measured at different zoom rates, 75%, 100%, 150%, 200%, and 300%.

myocyte areas for placebo heart are decreasing as the image zoom rate increases; (ii) from $\beta_2 + \beta_6 = 0.6$, $\beta_3 + \beta_7 = -3.0$, $\beta_4 + \beta_8 = -5.1$, and $\beta_5 + \beta_9 = -8.6$, we conclude that the means of the measured areas of myocytes of the treated heart are decreasing as the zoom rate increases above 150%; (iii) based on the Wald test statistics, β_2 and β_6 are not significantly different from 0, which indicate that the means of the measured areas of myocytes at zoom rates 75% and 100% are not significantly different; (iv) β_3 , β_4 , and β_5 are significantly

different from 0, which indicates that the measured areas of the myocytes from the placebo heart are significantly different across different zoom rates; (v) based on the estimate for β_2 , β_3 , β_4 , and β_5 , the mean change from zoom rate 100% to 150% is more dramatic than for any other pairs; (vi) β_7 , β_8 , and β_9 are not significantly different, which indicates that the differences of the means for treated heart and placebo heart are not significantly different at the three different zoom rates 150%, 200%, and 300%; and (vii) $\sigma_b^2 = 101.6^2$ and $\sigma^2 = 4.4^2$, which

Table 3 Expected values for different types of hearts at different zoom rates based on model (I)

| Zoom rate | 75% | 100% | 150% | 200% | 300% |
|-----------|---------------------|---|---|---|---|
| Placebo | β_0 | $\beta_0 + \beta_2$ | $\beta_0 + \beta_3$ | $\beta_0 + \beta_4$ | $\beta_0 + \beta_5$ |
| Treatment | $\beta_0 + \beta_1$ | $\beta_0 + \beta_1 + \beta_2 + \beta_6$ | $\beta_0 + \beta_1 + \beta_3 + \beta_7$ | $\beta_0 + \beta_1 + \beta_4 + \beta_8$ | $\beta_0 + \beta_1 + \beta_5 + \beta_9$ |

Table 4 The estimated parameters, their standard errors, the Wald-type *t*-test statistics, and *P*-values resulted from applying the linear mixed-effect model (1) to the current experimental data

| Parameters | Estimate | Standard errors | <i>t</i> -statistics | <i>P</i> -values |
|---------------------------------|----------|-----------------|----------------------|------------------|
| Intercept β_0 | 336.8 | 9.9 | 33.9 | 0.000 |
| Treatment β_1 | 45.5 | 13.9 | 3.3 | 0.001 |
| Zoom rate 100% β_2 | -1.1 | 0.8 | 1.4 | 0.164 |
| Zoom rate 150% β_3 | -9.6 | 0.7 | -13.9 | 0.000 |
| Zoom rate 200% β_4 | -11.9 | 0.7 | -17.6 | 0.000 |
| Zoom rate 300% β_5 | -14.6 | 0.7 | -20.0 | 0.000 |
| Treat: zoom rate 100% β_6 | 1.7 | 1.1 | 1.6 | 0.117 |
| Treat: zoom rate 150% β_7 | 6.6 | 1.0 | 6.9 | 0.000 |
| Treat: zoom rate 200% β_8 | 6.8 | 0.9 | 7.2 | 0.000 |
| Treat: zoom rate 300% β_9 | 6.0 | 1.0 | 5.9 | 0.000 |
| σ_b | 101.6 | | | |
| σ | 4.4 | | | |

indicates that the variances of the measurements are largely contributed by between-myocyte variation. This can also be seen from Figure 2, where the within-myocyte variation is much smaller than the between-myocyte variation.

Measurement accuracy and precision

Based on the *F*-test statistics for the equality of variances between two groups, the variances of the measurements of myocyte areas between the treated heart and the placebo heart are not significantly different at each zoom rate. Tables 2 and 4 show that the sample means seem to be decreasing when the image zoom rate increases for both the treated heart and the placebo heart. Let us define measurement accuracy as the sample mean, and define measurement precision as the sample variance.⁴ In the following paragraphs we will discuss statistical changes of accuracy and precision as image zoom rate increases.

The plot of the means of the measured areas versus zoom rates is shown in Figure 3. From Figure 3, it is clear that the means of measurements are approximately the same when the zoom rates are 75% and 100%, whereas the means of measured areas are decreasing as zoom rate increases, and the means of measured areas at zoom rates 150% and above are significantly smaller than those measured at zoom rate 100%. This decrease is reasonable because the measurement protocol is always trying to trace the inner edges, and the inner edge becomes clearer as the zoom rate increases. Therefore, this might indicate that measurement accuracy would increase as zoom rate increases. These findings are consistent with the findings based on the linear mixed-effect model (1). In addition, we performed paired *t*-tests for comparing the differences of measurements over any two image zoom rates for each type of heart. The results are presented

in Tables 5 and 6. Based on Tables 5 and 6, the measurements are not significantly different between zoom rates 75% and 100%, whereas all the other pairs are significantly different. The results hold for both the treated heart and the placebo heart. We conclude that measurement accuracy is increasing when the image zoom rate increases except between zoom rates at 75% and 100% for both the treated heart and the placebo heart.

In addition, we performed an *F*-test for the equality of variances between different zoom rates for each type of heart. All variances were not significantly different across different zoom rates and between the treated heart and the placebo heart. Therefore, it is concluded that with an increasing image zoom rate, measurement precision has no significant improvement.

The optimal zoom rate

Figure 4 depicts the *t*-statistics corresponding to different image zoom rates. Based on Figure 4, it seems that there is a big jump in the *t*-statistic from zoom rate 100% to zoom rate 150%, and the *t*-statistics remain at the same level among zoom rates 150%, 200%, and 300%.

Because all variances are not statistically different between different zoom rates in both the treated heart and the placebo heart, the pooled variances are not statistically different between any two different zoom rates on the condition of fixed sample sizes.

We examine whether the mean differences between the treated heart and the placebo heart over different zoom rates are significantly different. Denote $\Delta_{75} = \mu_{t75} - \mu_{p75}$, $\Delta_{100} = \mu_{t100} - \mu_{p100}$, $\Delta_{150} = \mu_{t150} - \mu_{p150}$, $\Delta_{200} = \mu_{t200} - \mu_{p200}$, and $\Delta_{300} = \mu_{t300} - \mu_{p300}$, where μ_{t75} is the mean area of the treated myocyte at zoom rate 75%, μ_{p100} is the mean area of the placebo myocyte at zoom

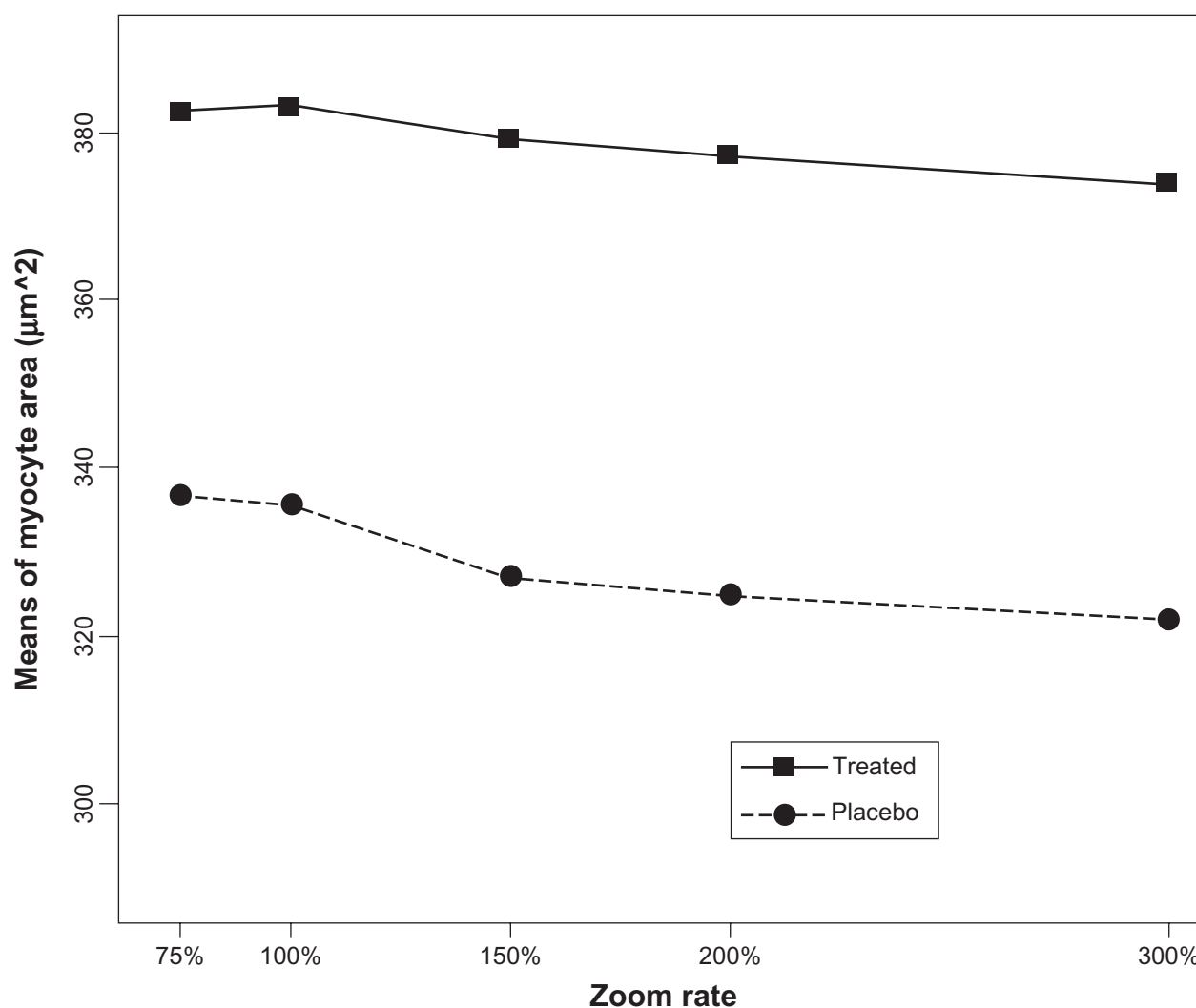


Figure 3 The means of measured myocyte areas over different zoom rates.

Table 5 Pairwise comparisons of means of treated myocytes at different zoom rates

| Comparison | P-value | Comparison | P-value |
|------------------------|---------|------------------------|---------|
| Zoom rate 75% vs 100% | 0.923 | Zoom rate 75% vs 200% | < 1e-04 |
| Zoom rate 75% vs 150% | 0.00015 | Zoom rate 75% vs 300% | < 1e-04 |
| Zoom rate 100% vs 150% | < 1e-04 | Zoom rate 150% vs 200% | < 1e-04 |
| Zoom rate 100% vs 200% | < 1e-04 | Zoom rate 150% vs 300% | < 1e-04 |
| Zoom rate 100% vs 300% | < 1e-04 | Zoom rate 200% vs 300% | < 1e-04 |

Table 6 Pairwise comparisons of means for placebo myocytes at different zoom rates

| Comparison | P-value | Comparison | P-value |
|------------------------|---------|------------------------|---------|
| Zoom rate 75% vs 100% | 0.616 | Zoom rate 75% vs 200% | < 1e-04 |
| Zoom rate 75% vs 150% | < 1e-04 | Zoom rate 75% vs 300% | < 1e-04 |
| Zoom rate 100% vs 150% | < 1e-04 | Zoom rate 150% vs 200% | < 1e-04 |
| Zoom rate 100% vs 200% | < 1e-04 | Zoom rate 150% vs 300% | < 1e-04 |
| Zoom rate 100% vs 300% | < 1e-04 | Zoom rate 200% vs 300% | < 1e-04 |

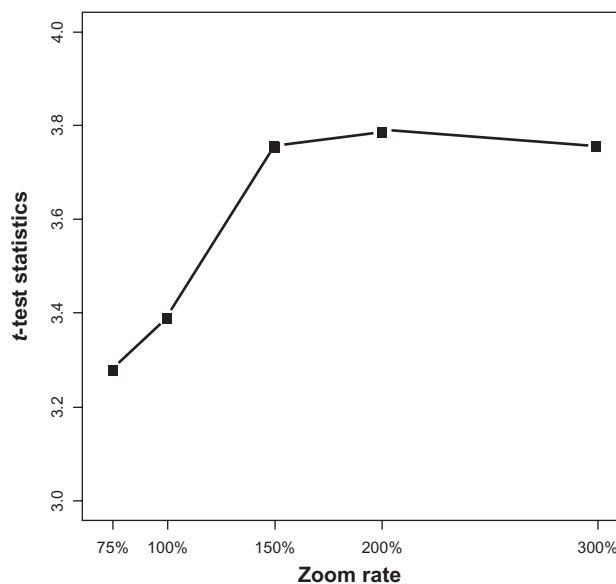


Figure 4 The t -test statistic for mean difference between the treated heart and the placebo heart at each zoom rate.

rate 75%, and the other quantities are similarly defined. To test whether the mean differences between the treated heart and the placebo heart over zoom rates 100% and 150% are significantly different, the underlying hypotheses are: $H_0: \Delta_{100} = \Delta_{150}$ versus $H_1: \Delta_{100} \neq \Delta_{150}$. The underlying hypotheses can be similarly defined when comparing the other pairs. We applied the t -test to test $H_0: \Delta_{100} = \Delta_{150}$ versus $H_1: \Delta_{100} \neq \Delta_{150}$. Because $\Delta_{150} - \Delta_{100} = (\mu_{t150} - \mu_{p150}) - (\mu_{t100} - \mu_{p100}) = (\mu_{t150} - \mu_{t100}) - (\mu_{p150} - \mu_{p100})$, the F -test for the equality of variances with the P -value of 0.787 indicates that the variances are equal between the measurements for $(\mu_{t150} - \mu_{t100})$ and $(\mu_{p150} - \mu_{p100})$. By using unpaired two-tailed t -tests with equal variances, the P -value is 3.48E-10, which indicates that there exists a significant difference between $(\mu_{t150} - \mu_{t100})$ and $(\mu_{p150} - \mu_{p100})$. There is a significant difference between Δ_{150} and Δ_{100} , and apparently $\Delta_{150} > \Delta_{100}$.

Similarly, we compared Δ_{200} and Δ_{150} . A P -value of 0.819 indicates that there is no significant difference between $(\mu_{t200} - \mu_{p150})$ and $(\mu_{p200} - \mu_{p150})$. There is no statistical difference between Δ_{200} and Δ_{150} , so we cannot reject the null hypothesis $H_0: \Delta_{150} = \Delta_{200}$. In addition, we compared Δ_{150} and Δ_{300} . A P -value of 0.342 indicates that we cannot reject the hypothesis $H_0: \Delta_{150} = \Delta_{300}$. It is concluded that, statistically, $\Delta_{150} > \Delta_{100}$, $\Delta_{150} > \Delta_{75}$, $\Delta_{150} = \Delta_{200}$, and $\Delta_{150} = \Delta_{300}$. Because there is no significant difference among the groups for the paired mean differences at different image zoom rates 150%, 200%, and 300%, the test statistic remains the same. However, the measurement cost, ie, the time spent to

trace the inner edges of these myocytes, is doubled with the zoom rate at 300% compared with that at 150%. Therefore, we conclude that the image zoom rate of 150% is the best to achieve the best test power and a low measurement cost.

Conclusion

As image zoom rate increases, measurement accuracy increases as well. However, measurement precision (variance) has no significant improvement.

Under current measurement conditions, after the image zoom rate reaches the threshold 150%, the mean difference remains a constant statistically. However, the measurement cost would increase dramatically as the image zoom rate increases. We can conclude that the image zoom rate 150% is optimal in terms of maximizing the test statistic with a low measurement cost.

Discussion

Because the measurement protocol is defined to trace the inner edge of myocytes, it is reasonable that accuracy will be increased as image zoom rate increases. However, Figure 2 indicates that most of the variations come from the between-myocyte differences for each type of heart, which may explain why the variances between different hearts across different zoom rates are not significantly different.

Achieving maximum accuracy and precision is the general goal in medical quantitative measurement. However, time and effort should also be considered at the current scientific and technical development level. All the measurements should be carried out in an affordable time. The conclusion drawn here may apply to other studies involving measuring area or length with instruments with adjusted zoom rates. The R -codes and the dataset are given in Appendix I and II, respectively.

Disclosure

The authors report no conflicts of interest in this work.

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Appendix I: R codes

```

data<-read.table(file.choose(),header=TRUE)
names(data)
#####333
### Raw data plot (Figure 2)
#####
par(mfrow=c(1,2), pty="m",mgp=c(1.3, 0.3,0),mai=c(0.6,0.6,0.6,0.1))
zoomrate<-c(75, 100, 150, 200, 300)
c.zr<-c("mt75", "mt100", "mt150", "mt200", "mt300")
c.treat<-c("placebo", "treatment")
ylim<-c(min(data$area), max(data$area))
y.bar<-rep(0, length(c.zr))
for (i in 1:2)
  { plot(c(75,300), ylim, type="n", xlab="Zoom Rate",
    ylab=paste("Readings for myocyte area"),
    main=paste(i, ". Measures for ",c.treat[i], sep=""))
    my.data<-data[data$treat==c.treat[i],]
    my.sub<-unique(my.data$myocyte)
    for (j in my.sub)
      {y<-my.data$area[my.data$myocyte==j]
        lines(zoomrate, y, lty=j, col=j)
      }
    for (j in 1:length(c.zr))
      {y.bar[j]<-mean(my.data$area[my.data$mt==c.zr[j]])}
      points(zoomrate, y.bar,pch=i+14, cex=1.5)
      lines(zoomrate, y.bar, lty=1, lwd=3)
    }
#####
model (1) in the manuscript
#####
par(mfrow=c(1,1), pty="m",mgp=c(1.3, 0.3,0),mai=c(0.6,0.6,0.6,0.1))
ID<-paste(data$treat, data$myocyte, sep="")
ord.ID<-order(ID)
data.ord<-data.frame(data[ord.ID,], ID=ID[ord.ID])
library(nlme)
data.ord2<-data.frame(area=data.ord$area,
  mt=factor(data.ord$mt,levels=c("mt75", "mt100", "mt150", "mt200", "mt300")),
  treat=data.ord$treat,ID=data.ord$ID)
longa<-groupedData(area~treat |ID, data=data.ord2)
fit.lme<-lme(area~treat*mt,data=longa,method="ML", random=~1)
summary(fit.lme1)
#####
##t-test for comparing measure type difference
#####
mt75trt<-data[data$mt=="mt75"&data$treat=="treatment",]
mt75placebo<-data[data$mt=="mt75"&data$treat=="placebo",]
qqnorm(mt75trt$area)
qqnorm(mt75placebo$area)

```



```

plot(density(mt75trt$area))
plot(density(mt75placebo$area))
var.test(mt75trt$area, mt75placebo$area)
t.test(mt75trt$area, mt75placebo$area, var.equal=TRUE)
sd(mt75trt$area)
sd(mt75placebo$area)
#####
### Figure 3
#####
names(data)
data$zoom.rate<-as.numeric(substring(data$mt, first=3, last=1000000L))
data.t<-data[data$treat=="treatment",]
data.c<-data[data$treat!="treatment",]
z.r<-c(75, 100,150, 200, 300)
mean.t<-mean.c<-rep(0,5)
for (i in 1:5)
{ mean.t[i]<-mean(data.t$area[data.t$zoom.rate==z.r[i]])
  mean.c[i]<-mean(data.c$area[data.c$zoom.rate==z.r[i]])
}
ymin<-min(mean.t,mean.c); ymax<-max(mean.t,mean.c)
par(mfrow=c(1,1),mgp=c(1.7, 0.5,0),mai=c(1.0,1.0,0.2,0.2))
plot(data$zoom.rate, data$area, xlim=c(70,300),ylim=c(290, 390),
xlab="Zoom Rate", ylab="Means of myocyte area (um^2)", cex.lab=1.5, type="n",xaxt="n")
axis(1, c(75, 100,150, 200, 300), c("75%", "100%", "150%", "200%", "300%"), cex=0.8)
points(z.r, mean.t, pch=15, cex=1.5)
lines(z.r, mean.t, lty=1, lwd=3)
points(z.r, mean.c, pch=16, cex=1.5)
lines(z.r, mean.c, lty=4, lwd=3)
legend(200, 310, legend=c("treated", "placebo"), lty=c(1,4), lwd=c(3,3),
      pch=c(15,16), cex=c(1.5, 1.5))
#####
## Figure 4
#####
t.stat<-rep(0,5)
#i<-1
for (i in 1:5)
{ d1<-data.t$area[data.t$zoom.rate==z.r[i]]
  d2<-data.c$area[data.c$zoom.rate==z.r[i]]
  t.stat[i]<-t.test(d1,d2,var.equal=TRUE)$statistic
}
par(mfrow=c(1,1),mgp=c(1.6, 0.5,0),mai=c(1.0,1.0,0.2,0.2))
plot(z.r, t.stat, xlim=c(70,300),ylim=c(3, 4),
xlab="Zoom Rate", ylab="t-test statistics", type="b", pch=15,lty=1,
lwd=3, cex=1.3, yaxt="n", cex.lab=1.5)
axis(1, c(75, 100,150, 200, 300), c("75%", "100%", "150%", "200%", "300%"), cex=0.8)
#####
##      END
#####

```

Appendix II: Dataset samples

| area | mt | treat | myocyte |
|---------|-------|-----------|---------|
| 259.509 | mt75 | treatment | 1 |
| 311.831 | mt75 | treatment | 2 |
| 255.403 | mt75 | treatment | 3 |
| 467.642 | mt75 | treatment | 4 |
| 300.672 | mt75 | treatment | 5 |
| 323.517 | mt75 | treatment | 6 |
| 251.297 | mt75 | treatment | 7 |
| 441.744 | mt75 | treatment | 8 |
| 341.098 | mt75 | treatment | 9 |
| 507.437 | mt75 | treatment | 10 |
| 432.690 | mt75 | treatment | 11 |
| 288.144 | mt75 | treatment | 12 |
| 376.366 | mt75 | treatment | 13 |
| 351.310 | mt75 | treatment | 14 |
| 465.010 | mt75 | treatment | 15 |
| 340.783 | mt75 | treatment | 16 |
| 549.969 | mt75 | treatment | 17 |
| 280.143 | mt75 | treatment | 18 |
| 402.054 | mt75 | treatment | 19 |
| ... | ... | ... | ... |
| 314.569 | mt300 | placebo | 94 |
| 327.307 | mt300 | placebo | 95 |
| 362.786 | mt300 | placebo | 96 |
| 286.670 | mt300 | placebo | 97 |
| 229.189 | mt300 | placebo | 98 |
| 361.101 | mt300 | placebo | 99 |
| 535.967 | mt300 | placebo | 100 |
| 468.484 | mt300 | placebo | 101 |
| 228.346 | mt300 | placebo | 102 |
| 514.595 | mt300 | placebo | 103 |
| 315.306 | mt300 | placebo | 104 |
| 377.946 | mt300 | placebo | 105 |
| 379.946 | mt300 | placebo | 106 |

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