Online Microscope Images Toolkit

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Veterinary Integrative Biosciences
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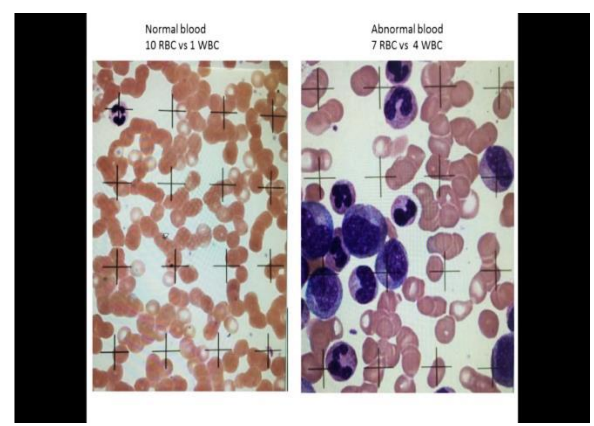
YouTube video of toolkit
http://www.youtube.com/vibshistology

Toolkit Contents

Why do cell counts (stereology)?

Stereology (random independent sampling of the whole group as opposed to counting all individuals in that group) allows quantitative analyses of images, cells, tissues, or micrographs to supplement general observations. It tells you how much change occurred not just that a change has occurred. This might indicate how sick a person is, how successful a treatment might be, or whether a person is getting better or worse. It might be used to determine if an observed change is statistically significant.



Why do stereology and integrate technology and math (STEM) in science?

Use of this toolkit gives students an opportunity to evaluate microscopic images where they interact with images of specimens (each student makes his/her own measurements) and use technology to do so as they integrate math and science. They see the value of and use math in scientific investigation to determine the amount of difference between samples. One might hypothesize that abnormal blood has 10 to 100 times the concentration of white blood cells as does the normal blood, but does the conclusion of the stereological analyses agree?

Cell Count

	Healthy Blood		Unhealthy Blood	
	RBC	WBC	RBC	WBC
Total Cells Counted	X	X	X	X

Cell Volume Density

	Healthy Blood		Unhealthy Blood	
	RBC	WBC	RBC	WBC
Total Hits	x	x	x	x

Calculations/ technology

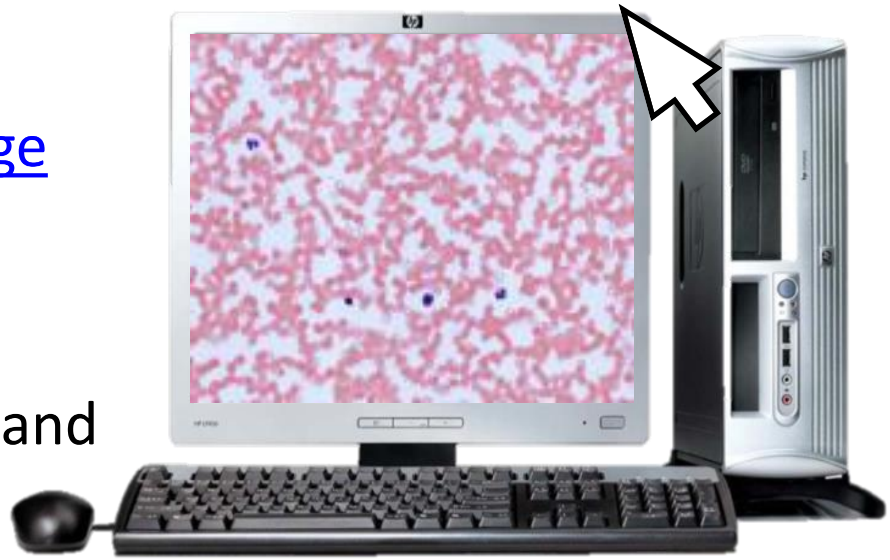
1. Operating Microscope Images

Online Microscope Image Links:

[Healthy Blood Smear Image]

[Unhealthy Blood Smear Image]

[Human Testes Image]



To move around the image, click and drag with the mouse to move to different parts of the image.

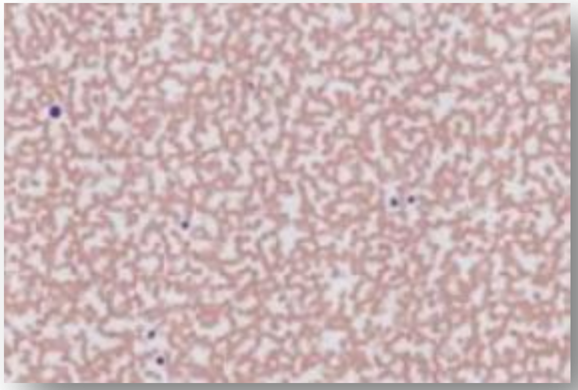
To move quickly to a completely new area, click at one corner and drag to the opposite corner.

Note: These images are taken from dead but preserved cells and tissues which were stained to allow observation and digitizing by light or electron microscopy.

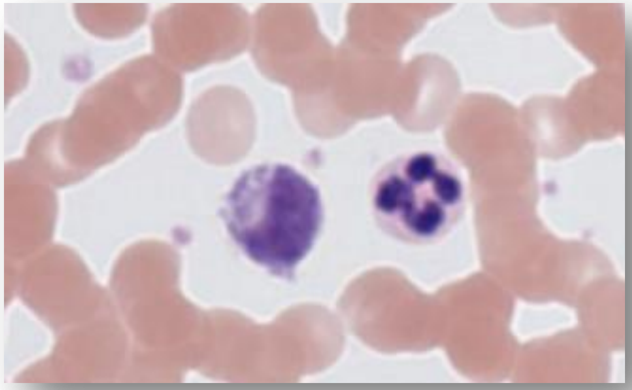
Return to Toolkit

1. Operating Microscope Images

To change Magnification, click the magnifying glass icons at the top left tool bar or use the mouse scroll wheel. When scrolling, the different magnifications are indicated above the word "Magnification."



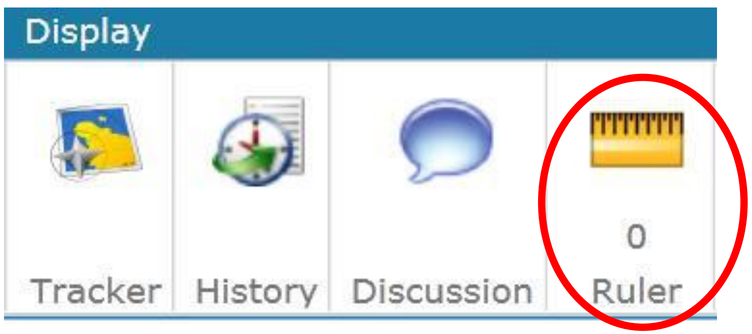
5x



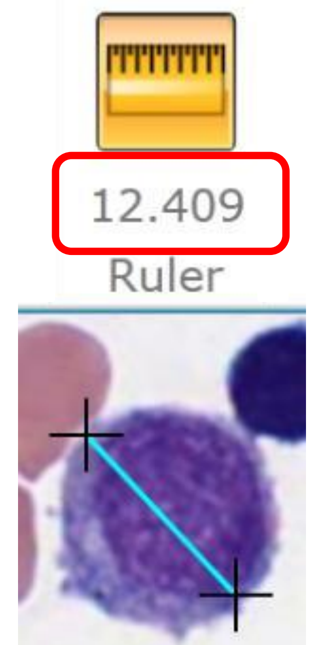
80x

1. Operating Microscope Images

To turn on the Ruler tool, click on the ruler icon in the top toolbar.



Place your arrow on one side of the cell, click and drag your pointer to the other side, and click to measure a cell's diameter.



Values are displayed beneath in micrometers (µm).

To turn off the ruler tool, click the ruler icon again.

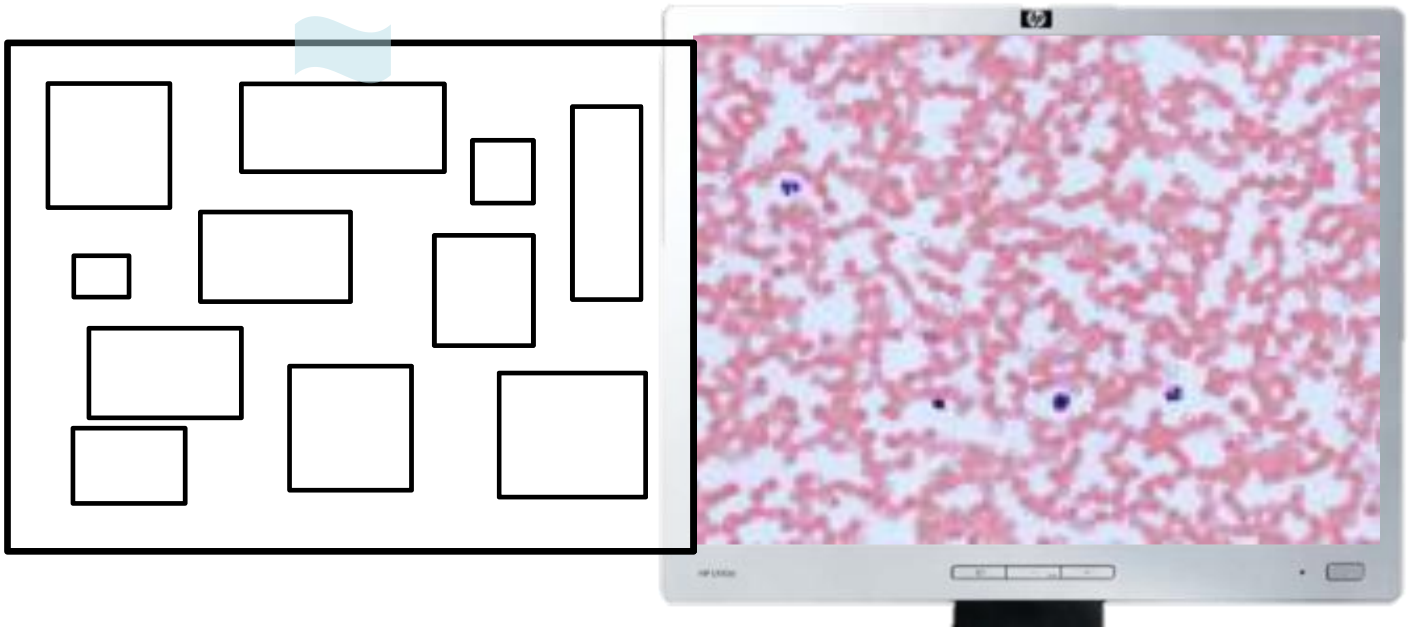
Note, you must turn off the ruler to change magnification or move to a different area.

2. Cell Count Procedure

A. Print out the Box Counter on a transparency sheet.

B. Set the magnification to 80x.

C. Hold the Box Counter transparency over the computer screen. Tape it in place if need be.



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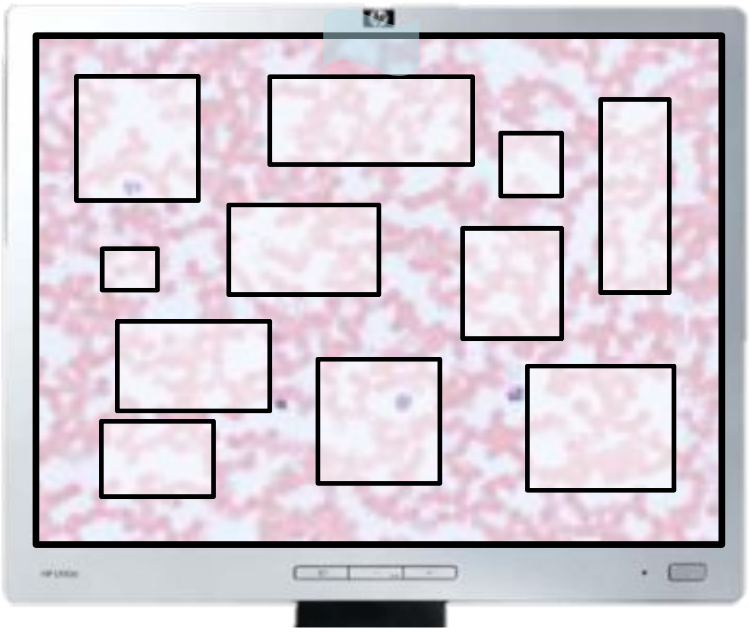
2. Cell Count Procedure

D. Students will do a separate count for red blood cells and white blood cells.

E. Count the number of cells inside each box, including ones touching the boxes borders to provide <u>a random sampling that represents the whole.</u>

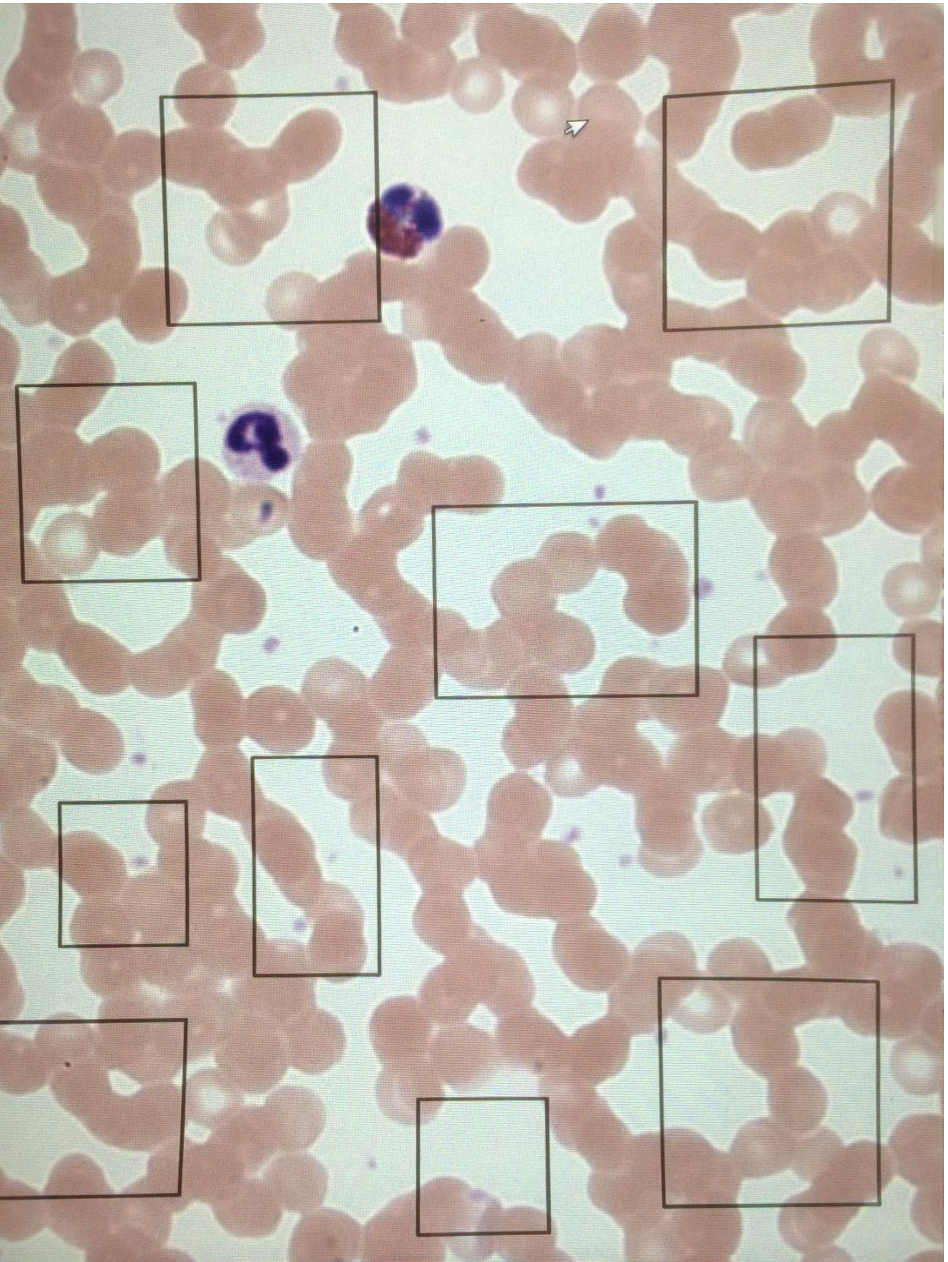
F. Record the numbers in the worksheet and add up totals.

G. Move to a new area and repeat two or more times.

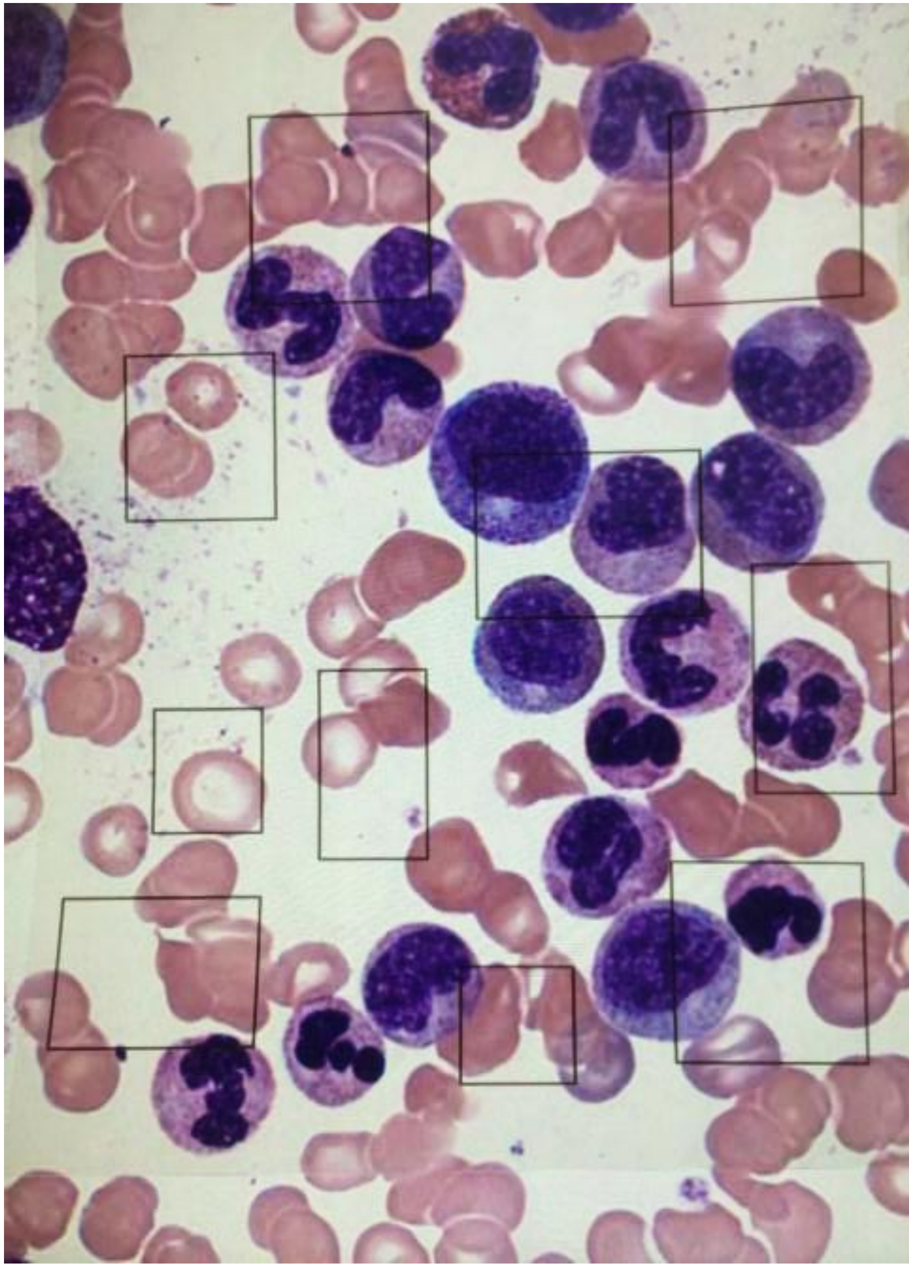


<u>Note: stacked overlapped red blood cells</u>
Regarding stacked overlapped blood cells in the abnormal blood sample, the best we can do is to count the curved lines (edges of cells) in a stacked group and an estimate here is required.

Normal blood
127 RBC vs 1 WBC



Abnormal blood
72 RBC vs 14 WBC

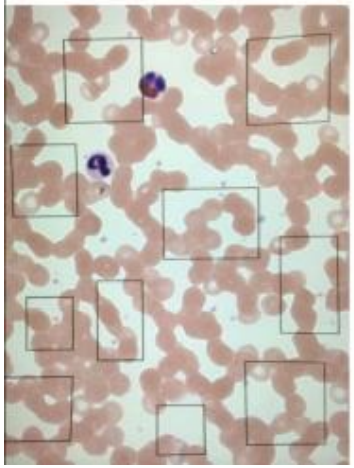


2. Cell Count - Example Data

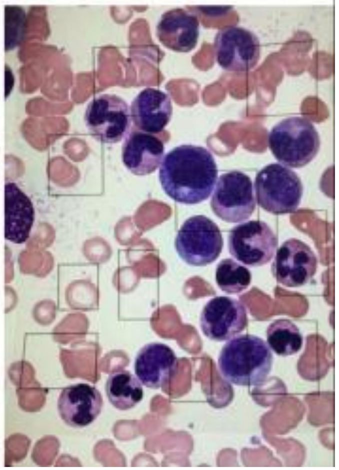
Healthy Blood		Unhealthy Blood	
RBC	WBC	RBC	WBC
28	0	8	10
15	1	15	7
25	0	5	6
23	0	14	4
30	1	8	5
…	…	…	…
…	…	…	…
40	1	6	5

Total Cells Counted	**633**	**2**	**237**	**62**

Normal blood
127 RBC vs 1 WBC



Abnormal blood
72 RBC vs 14 WBC



Ratio of cells
Magnitude of difference

2/ 633 = .003 **62/ 237 = .262**

.262/ .003 = 87 times

Conclusion : white blood cells are 87 times more concentrated in the abnormal blood.

To determine the significance:

Finding the Ratios

Healthy Blood RBC/WBC	Ratio	Unhealthy Blood RBC/WBC	Ratio
15/1	= 15	10/9	= 1.11
17/1	= 17	7/8	= 0.88
21/1	= 21	10/4	= 2.5
21/1	= 21	8/12	= 0.66
21/2	= 10.5	10/5	= 2.0
19/1	= 19	5/5	= +1
	103.5/6= 17.25		8.15/6= 1.36

These ratios compare number of times a RBC or a WBC was touched by the cross. If these numbers are added up and divided by the number of data collections (6) then the answer is the average, or **MEAN**

The **MEAN** of these numbers is used to find the standard deviation, or **σ**. The standard deviation measures how spread out the numbers are.

Finding the Variance

Healthy Blood RBC / WBC		Unhealthy Blood RBC / WBC	
15/1	= 15	10/9	= 1.11
17/1	= 17	7/8	= 0.88
21/1	= 21	10/4	= 2.5
21/1	= 21	8/12	= 0.66
21/2	= 10.5	10/5	= 2.0
19/1	= +19	5/5	= +1
Mean	**17.25**		**1.36**

Standard Deviation is the square root of the **VARIANCE.** To find the variance, the mean is subtracted from each number, and the result is squared. Then find the mean of these numbers.

15 – 17.25 = -2.25^2 = 5.06
17 – 17.25 = -0.25^2 = 0.06
21 – 17.25 = 3.75^2 = 14.06
21 – 17.25 = 3.75^2 = 14.06
10.5 – 17.25 = -6.75^2 = 45.56
19 – 17.25 = 1.75^2 = 3.06
81.86/6=
13.64

1.11 – 1.36 = -0.25^2 = 0.06
0.88 – 1.36 = -0.48^2 = 0.23
2.50 – 1.36 = 1.14^2 = 1.30
0.66 – 1.36 = -0.70^2 = 0.49
2.0 – 1 .36 = 0.64^2 = 0.41
1.0 - 1.36 = -0.36^2 = 0.13
2.62/6=
0.44

Finding the Standard Deviation

Standard Deviation is the square root of the **VARIANCE.**

	Healthy Blood		Unhealthy Blood
	RBC / WBC		RBC / WBC
	15/1 = 15		10/9 = 1.11
	17/1 = 17		7/8 = 0.88
	21/1 = 21		10/4 = 2.5
	21/1 = 21		8/12 = 0.66
	21/2 = 10.5		10/5 = 2.0
	19/1 = 19		5/5 = +1
Mean	**17.25**		**1.36**
Variance	**13.64**		**0.44**
Standard Deviation	**3.69**		**0.66**

Standard Deviation

√13.64 = **3.69**

√0.44 = **0.66**

Explanation of terms

n = The sample size
$\bar{x}$ = The mean of a sample
s = The standard deviation
var = The variance (equal to s^2)

The standard deviation (s) can be calculated using the formula:

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

Doing a T Test

Explanation of terms

n = The sample size
$\overline{x}$ = The mean of a sample
s = The standard deviation
var = The variance (equal to s^2)

The standard deviation (s) can be calculated using the formula:

$$s = \sqrt{\frac{\sum (x-\overline{x})^2}{n-1}}$$

A **T Test** determines if the difference between two means is significant. This T Test will compare the means of the nuclei data in plant and animal cells.

Two-sample t-test

To test whether the mean of a sample ($\overline{x}_1$) differs significantly from the mean of another sample ($\overline{x}_2$)...

Calculate the 'standard error of the mean' (SEM):

$$SEM = \sqrt{\frac{\mathrm{var}_1}{n_1} + \frac{\mathrm{var}_2}{n_2}}$$

Calculate the t-statistic:

$$t = \frac{\overline{x}_1 - \overline{x}_2}{SEM}$$

Use the table of critical values to find out whether or not the result is significant.

$$SEM = \sqrt{\frac{13.64}{6} + \frac{0.44}{6}} = 1.53$$

$$t = \frac{17.25 - 1.36}{1.53} = 10.39$$

Doing a T Test

$$t = \frac{17.25 - 1.36}{1.53} = 10.39$$

Table of critical values

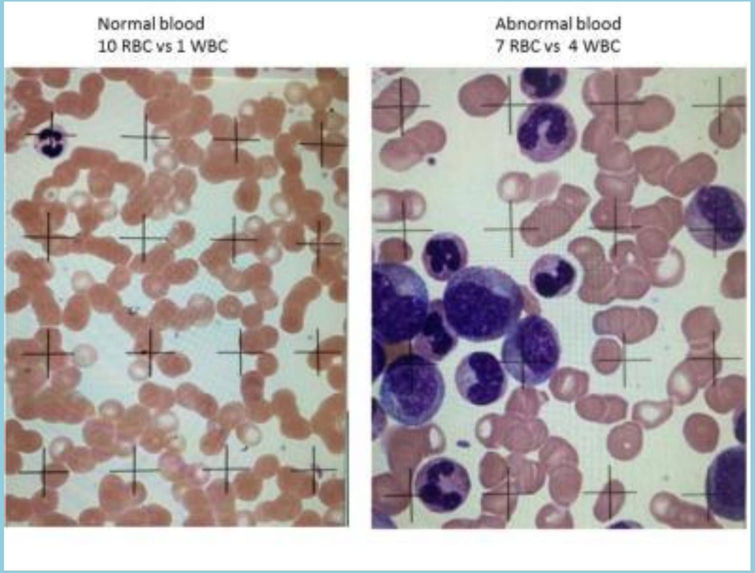
The table below gives the t-value at which the result has a particular level of 'significance'.

d.f. is the number of 'degrees of freedom'. In this case, *d.f.* = *n* -1
(If the exact *d.f.* value that you want is not included in the table, use the closest value below it that is included.)
p is the probability that the difference between two samples, or the difference between a sample and the theoretical result, is entirely due to chance.

d.f.	p=0.1	p=0.05	p=0.01
2	2.92	4.30	9.92
3	2.35	3.18	5.84
4	2.13	2.78	4.60
5	2.02	2.57	4.03
6	1.94	2.45	3.71
7	1.89	2.36	3.50
8	1.86	2.31	3.36
9	1.83	2.26	3.25
10	1.81	2.23	3.17

D.f. = 6-1 = 5

Since t = 10.39
10.39 > 4.03



$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

Since 10.39 is greater than 4.03, the difference between the ratios of RBC to WBC in the normal and abnormal blood sample is significant at the p=0.01 level.

$$SEM = \sqrt{\frac{\mathrm{var}_1}{n_1} + \frac{\mathrm{var}_2}{n_2}}$$

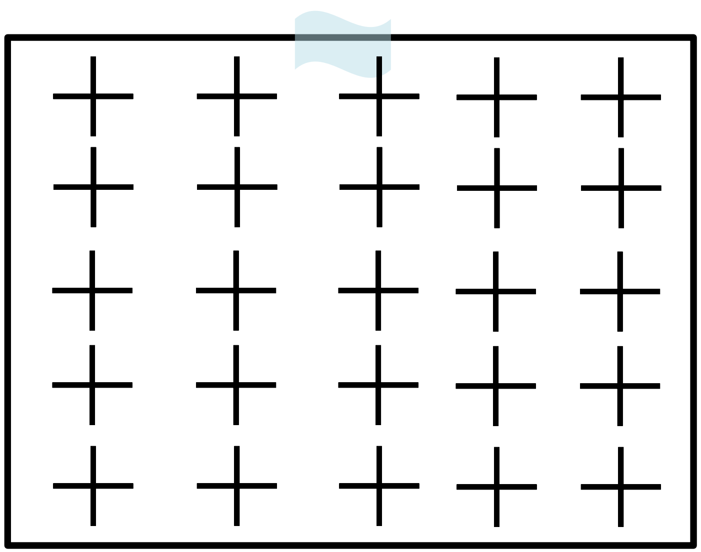
$$t = \frac{\bar{x}_1 - \bar{x}_2}{SEM}$$

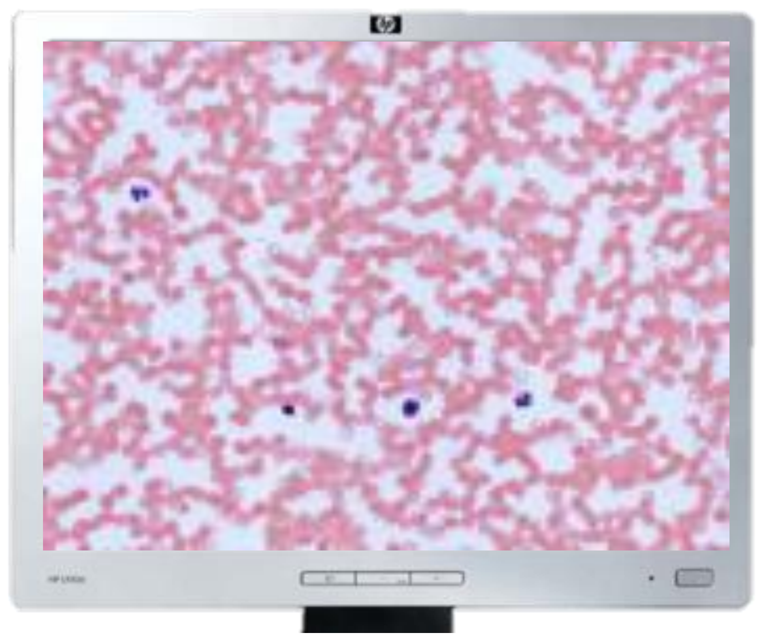
3. Cell Volume Density

A. Print out the Plus Counter on a transparency sheet.

B. Open the healthy blood image, and set the magnification to 80x.

C. Hold the Plus Counter transparency over the computer screen. Tape it in place if need be.





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3. Cell Volume Density

D. Count the number of cells under the center of each cross (one "hit" is scored for each cross) as <u>a random sampling that represents the whole.</u>

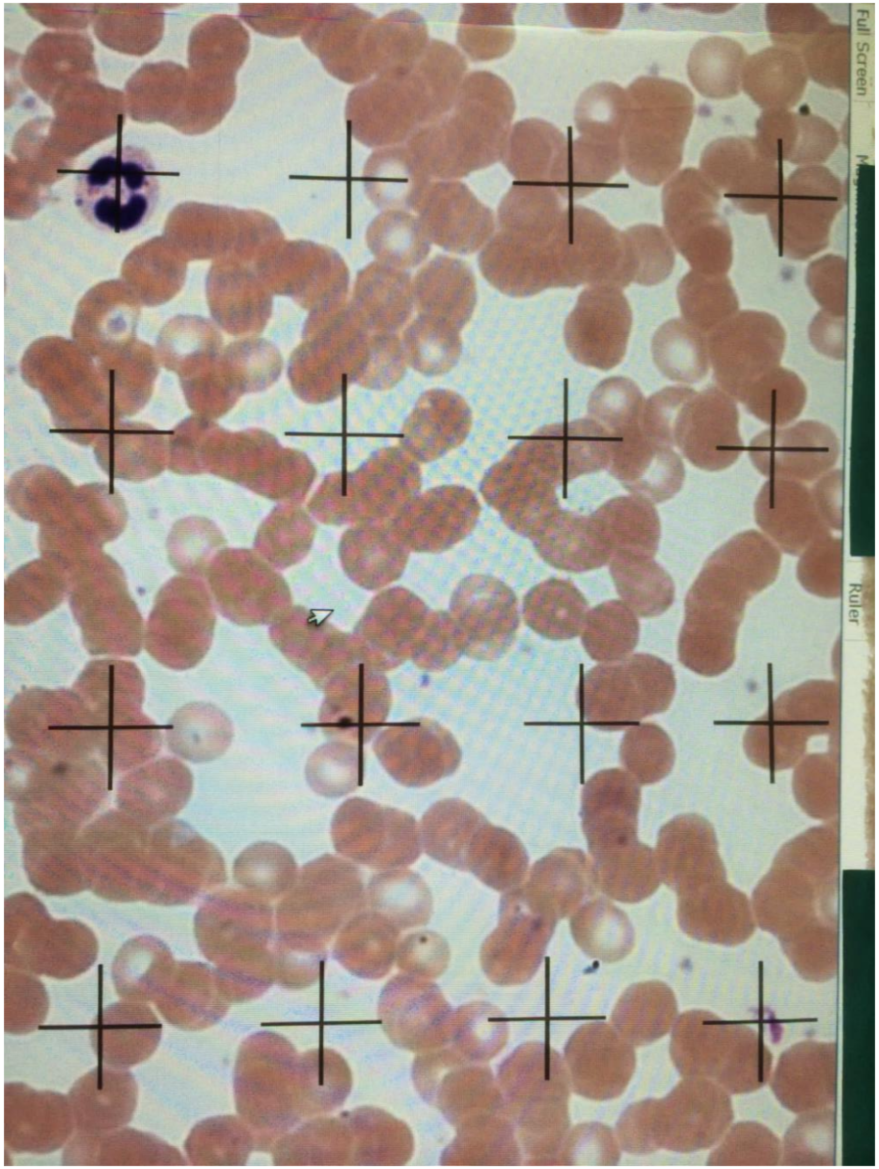
E. Record the numbers in the worksheet and add up totals.

F. Move to a new area and repeat hit count 5 or more times.

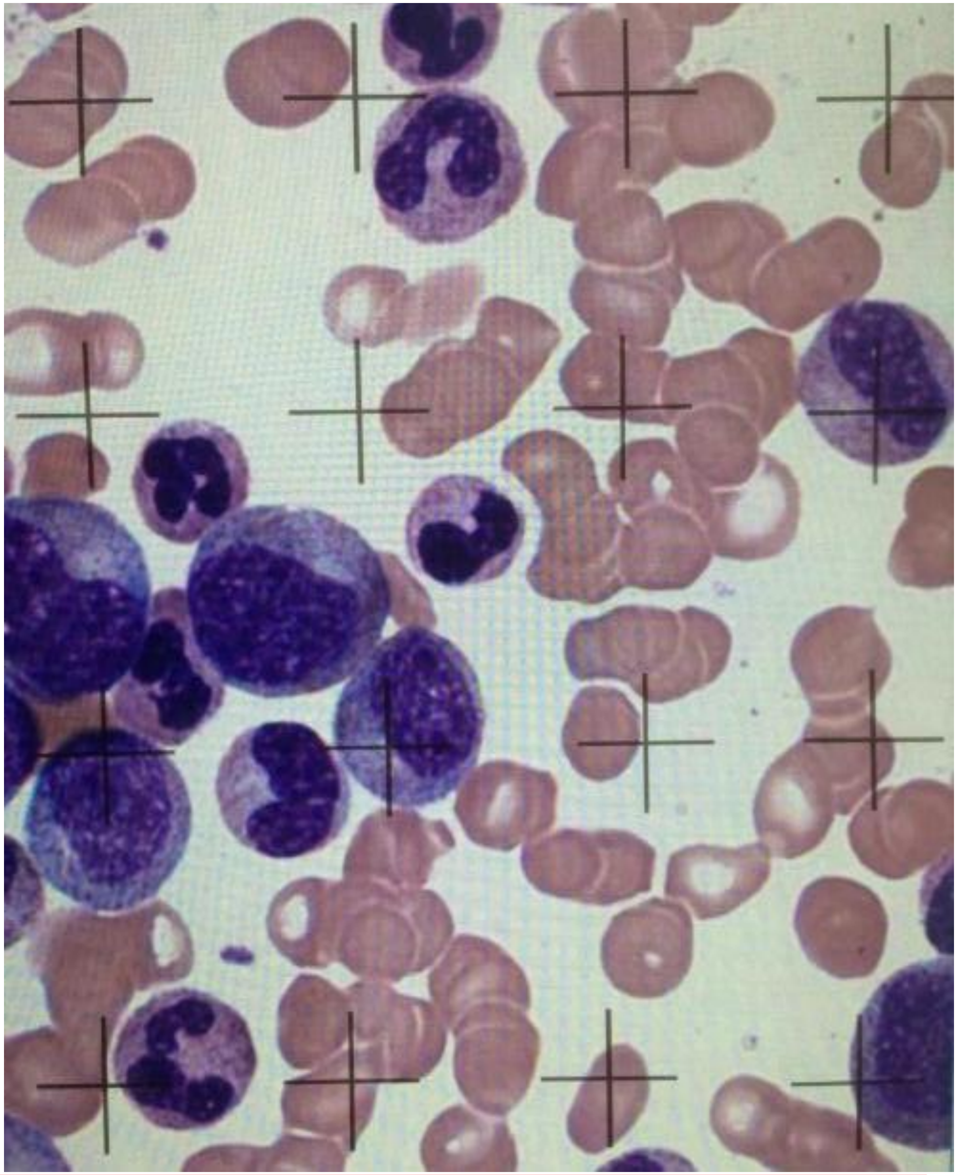
G. Add up the total hits that land on red blood cells from all repeated counts, divide by the number of possible "hits" (e.g., 5 repeats X 25 pluses per sheet = 125), and multiply that value by 100 to calculate cell volume density.

H. Repeat for white blood cells.

Normal blood
10 RBC vs 1 WBC



Abnormal blood
7 RBC vs 4 WBC

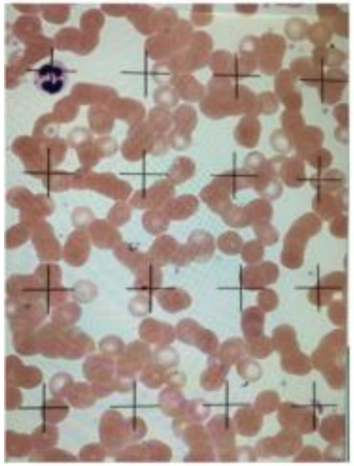


Note: To show cells at higher magnification, only 16 of the 25 crosses are shown here

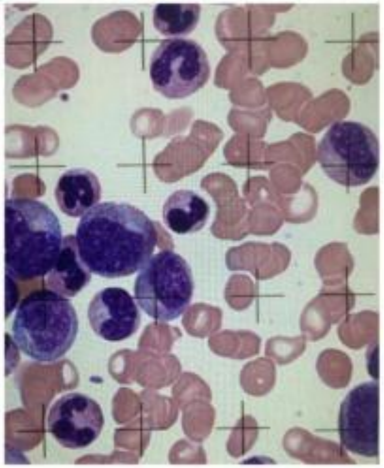
3. Cell Volume Density Example Data

	Healthy Blood		Unhealthy Blood	
	RBC	WBC	RBC	WBC
	14	0	10	9
	16	0	7	8
	20	0	10	4
	20	0	8	12
	20	1	10	5
	...	...	...	...
	...	...	...	...
	18	0	5	5
Total Hits	**274**	**1**	**133**	**92**

Normal blood
10 RBC vs 1 WBC



Abnormal blood
7 RBC vs 4 WBC



Ratio of cells
Magnitude of difference

1/ 274 = .0036 92/133 = .6917

.6917/.0046 = 150 times

Conclusion : white blood cells occupy 150 times more volume in the abnormal blood.
Is this statistically significant?

Do analyses from both the cell count (boxes) and volume density (crosses) conclude similar results (magnitude of difference)?

Total Cells Counted

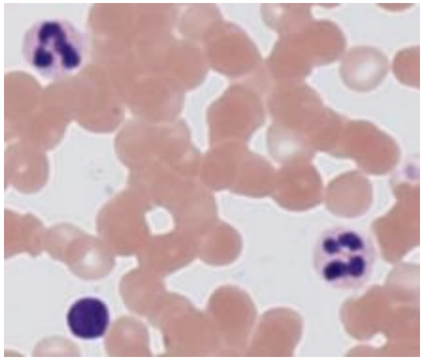
633	2	237	62

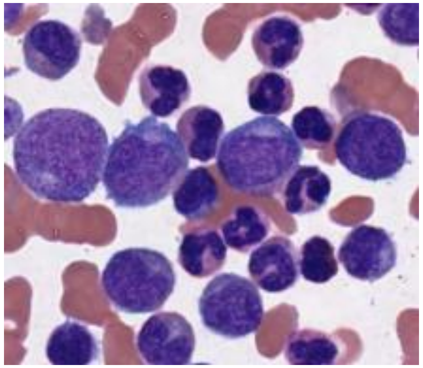
Ratio of cells Magnitude of difference

2/ 633 = .003 **62/ 237 = .262**

.262/ .003 = 87 times

Conclusion : white blood cells are 87 times more concentrated in the abnormal blood.





Ratio of cells Magnitude of difference

1/ 274 = .0036 **92/133 = .6917**

.6917/.0046 = 150 times

Conclusion : white blood cells occupy 150 times more volume in the abnormal blood.
Is this statistically significant?

Conclusions: both methods indicated a larger ratio of white blood cells in abnormal blood; however, the greater magnitude of difference estimated by volume density indicates that the size of individual white blood cells were larger in the abnormal blood.

3. Cell Volume Density Example Data:

percentage of whole occupied by each component

	Healthy Blood		Unhealthy Blood	
Total Hits	**274**	**1**	**133**	**92**
	RBC	WBC	RBC	WBC
Total Cells Counted	274 + 1 = **275**		133 + 92 = **225**	

Volume Density of Cells

Healthy Blood

RBC: $\frac{274}{275} \times 100 = \boxed{\mathbf{99\%}}$

WBC: $\frac{1}{275} \times 100 = \boxed{\mathbf{0.36\%}}$

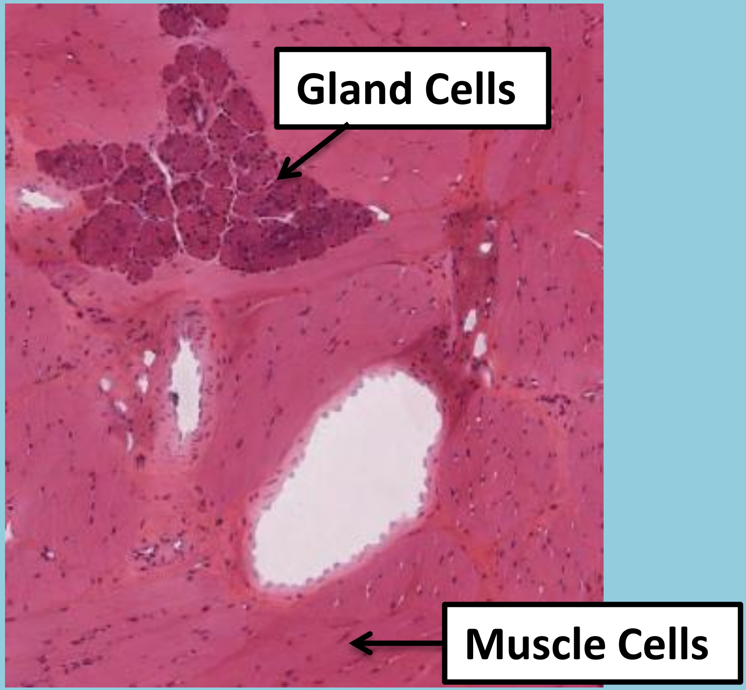
Unhealthy Blood

RBC: $\frac{133}{225} \times 100 = \boxed{\mathbf{59\%}}$

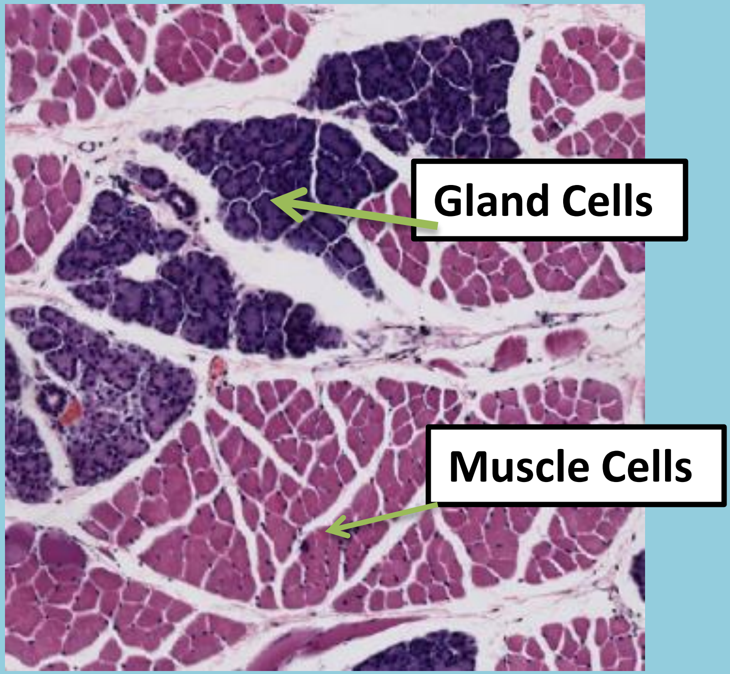
WBC: $\frac{92}{225} \times 100 = \boxed{\mathbf{40\%}}$

Comparing the Gland to Muscle ratio in the Monkey Tongue and the Rabbit Tongue

Monkey



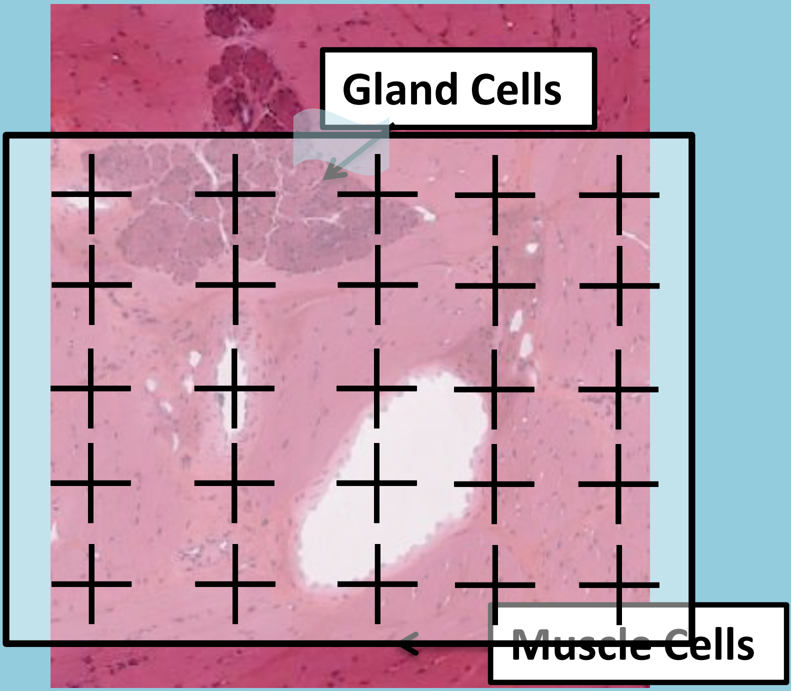
Rabbit

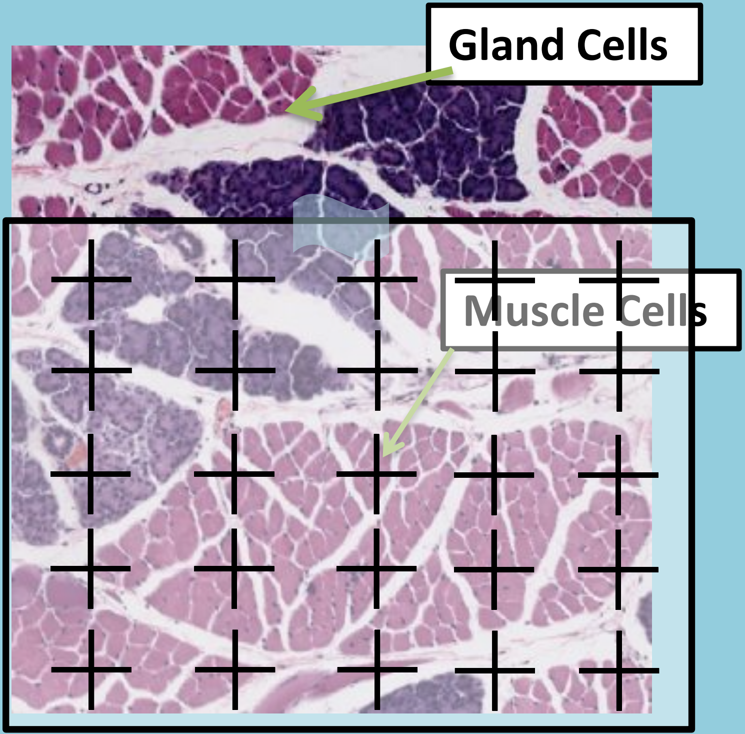


- There are both Glandular Cells and Muscle Cells in the tongue of both species.

Comparing the amount of Glandular Cells and Muscle Cells

- By using the print out on the computer screen, you can get data about the ratio for glandular cells to muscle cells





To determine the significance:

Finding the Ratios

Monkey Tongue		Rabbit Tongue	
gland / muscle	Ratio	gland / muscle	Ratio
4/67	= 0.06	3/52	= 0.06
4/66	= 0.06	2/45	= 0.04
3/72	= 0.04	2/46	= 0.04
4/67	= 0.06	2/52	= 0.04
3/70	= 0.04	1/54	= 0.02
	0.26/5=		0.20/5=
	0.052		0.04

These ratios compare number of times a Gland or a Muscle cell was touched by the cross. If these numbers are added up and divided by the number of data collections (5) then the answer is the average, or **MEAN**

The **MEAN** of these numbers is used to find the standard deviation, or **σ**. The standard deviation measures how spread out the numbers are.

Finding the Variance

Monkey Tongue		Rabbit Tongue	
gland/muscle	Ratio	gland/muscle	Ratio
4/67	= 0.06	3/52	= 0.06
4/66	= 0.06	2/45	= 0.04
3/72	= 0.04	2/46	= 0.04
4/67	= 0.06	2/52	= 0.04
3/70	= 0.04	1/54	= 0.02
Mean	**0.052**		**0.040**

Standard Deviation is the square root of the **VARIANCE.** To find the variance, the mean is subtracted from each number, and the result is squared. Then find the mean of these numbers.

$0.06 - 0.052 = 0.008^2 = 0.000064$
$0.06 - 0.052 = 0.008^2 = 0.000064$
$0.04 - 0.052 = -0.012^2 = 0.000144$
$0.06 - 0.052 = 0.008^2 = 0.000064$
$0.04 - 0.052 = -0.012^2 = 0.000144$
$=0.000544/5$
0.0001088

$0.06 - 0.040 = 0.02^2 = 0.0004$
$0.04 - 0.040 = 0.00^2 = 0.0000$
$0.04 - 0.040 = 0.00^2 = 0.0000$
$0.04 - 0.040 = 0.00^2 = 0.0000$
$0.02 - 0.040 = -0.02^2 = 0.0004$
$0.0008/5=$
0.00016

Finding the Standard Deviation

Standard Deviation is the square root of the **VARIANCE.**

Monkey Tongue gland / muscle	Ratio	Rabbit Tongue gland / muscle	Ratio
5/20	= 0.25	5/13	= 0.38
2/22	= 0.09	8/7	= 1.14
7/14	= 0.50	9/7	= 1.29
3/20	= 0.15	10/7	= 1.43
2/22	= 0.09	6/11	= 0.55
Mean	**0.052**		**0.96**
Variance	**0.0001088**		**0.00016**
Standard Deviation	**0.0104**		**0.0126**

Standard Deviation

$\sqrt{0.0001088}$ = **0.0104**

$\sqrt{0.00016}$ = **0.0126**

Explanation of terms

n = The sample size
$\bar{x}$ = The mean of a sample
s = The standard deviation
var = The variance (equal to s^2)

The standard deviation (s) can be calculated using the formula:

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

Doing a T Test

Explanation of terms

n = The sample size
$\bar{x}$ = The mean of a sample
s = The standard deviation
var = The variance (equal to s^2)

The standard deviation (s) can be calculated using the formula:

$$s = \sqrt{\frac{\sum (x-\bar{x})^2}{n-1}}$$

A **T Test** determines if the difference between two means is significant. This T Test will compare the means of the nuclei data in plant and animal cells.

Two-sample t-test

To test whether the mean of a sample ($\bar{x}_1$) differs significantly from the mean of another sample ($\bar{x}_2$)...

Calculate the 'standard error of the mean' (SEM):

$$SEM = \sqrt{\frac{var_1}{n_1} + \frac{var_2}{n_2}}$$

Calculate the t-statistic:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{SEM}$$

Use the table of critical values to find out whether or not the result is significant.

$$SEM = \sqrt{\frac{0.0001088}{5} + \frac{0.00016}{5}} = 0.0073$$

$$t = \frac{0.22 - 0.96}{0.29} = -2.55$$

Doing a T Test

Table of critical values

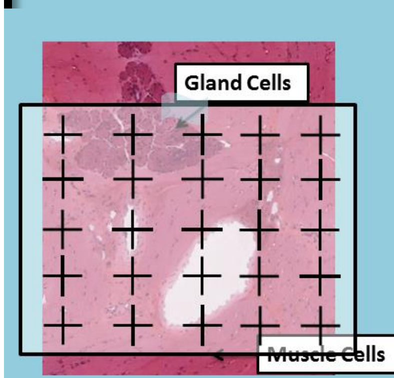
The table below gives the t-value at which the result has a particular level of 'significance'.

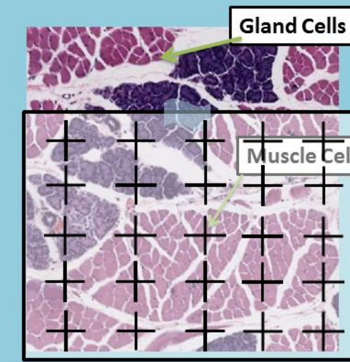
d.f. is the number of 'degrees of freedom'. In this case, *d.f.* = *n* -1
(If the exact *d.f.* value that you want is not included in the table, use the closest value below it that is included.)
p is the probability that the difference between two samples, or the difference between a sample and the theoretical result, is entirely due to chance.

d.f.	p=0.1	p=0.05	p=0.01
2	2.92	4.30	9.92
3	2.35	3.18	5.84
4	2.13	2.78	4.60
5	2.02	2.57	4.03
6	1.94	2.45	3.71
7	1.89	2.36	3.50
8	1.86	2.31	3.36
9	1.83	2.26	3.25
10	1.81	2.23	3.17

D.f. = 5-1 = 4

Since t = 2.55
2.13<2.55<2.78





$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

Since 2.55 is less than the value for p=0.05, the difference is not statistically significant. However, since the value is greater than the value for p = 0.10, the test shows a trend.

$$SEM = \sqrt{\frac{\mathrm{var}_1}{n_1} + \frac{\mathrm{var}_2}{n_2}}$$

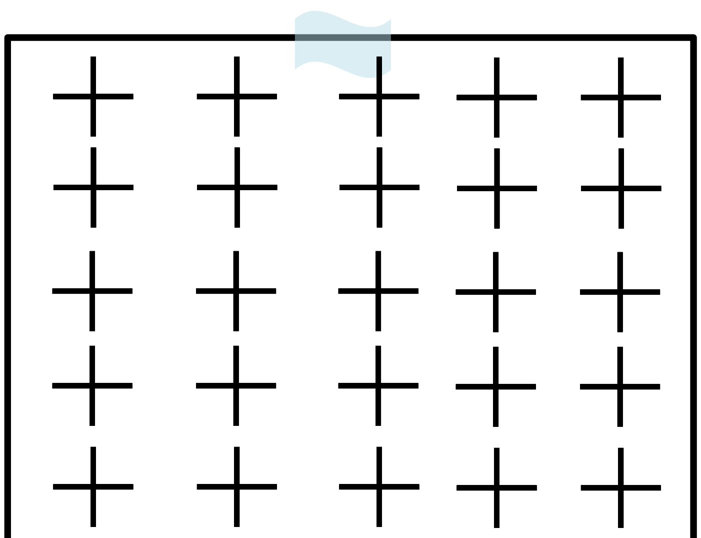
$$t = \frac{\bar{x}_1 - \bar{x}_2}{SEM}$$

3. (Cont'd) Tissue Volume Density

A. Print out the Plus Counter on a transparency sheet.

B. Open the human testes online image to 10x magnification.

C. Hold the Plus Counter transparency over the computer screen. Tape it in place if need be.

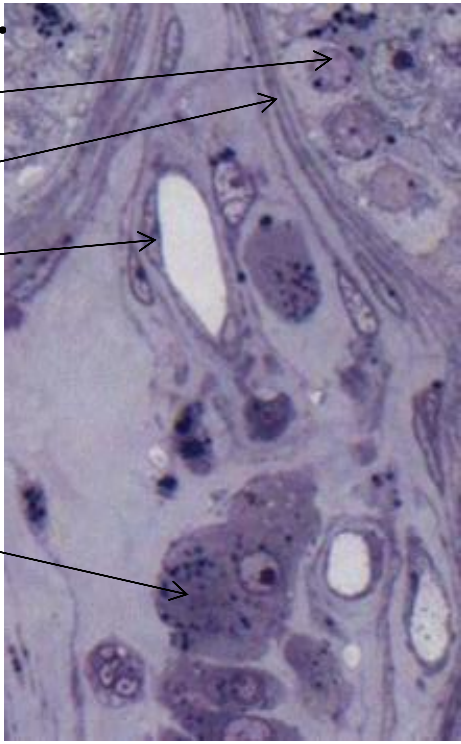


Return to Toolkit

3. Tissue Volume Density

D. Count the number of structures under the center of each cross (one "hit" is scored for each cross).

E. Structure will be either: Seminiferous Tubules, Boundary Tissue of Seminiferous Tubules, Leydig Cells, or Blood Vessels.

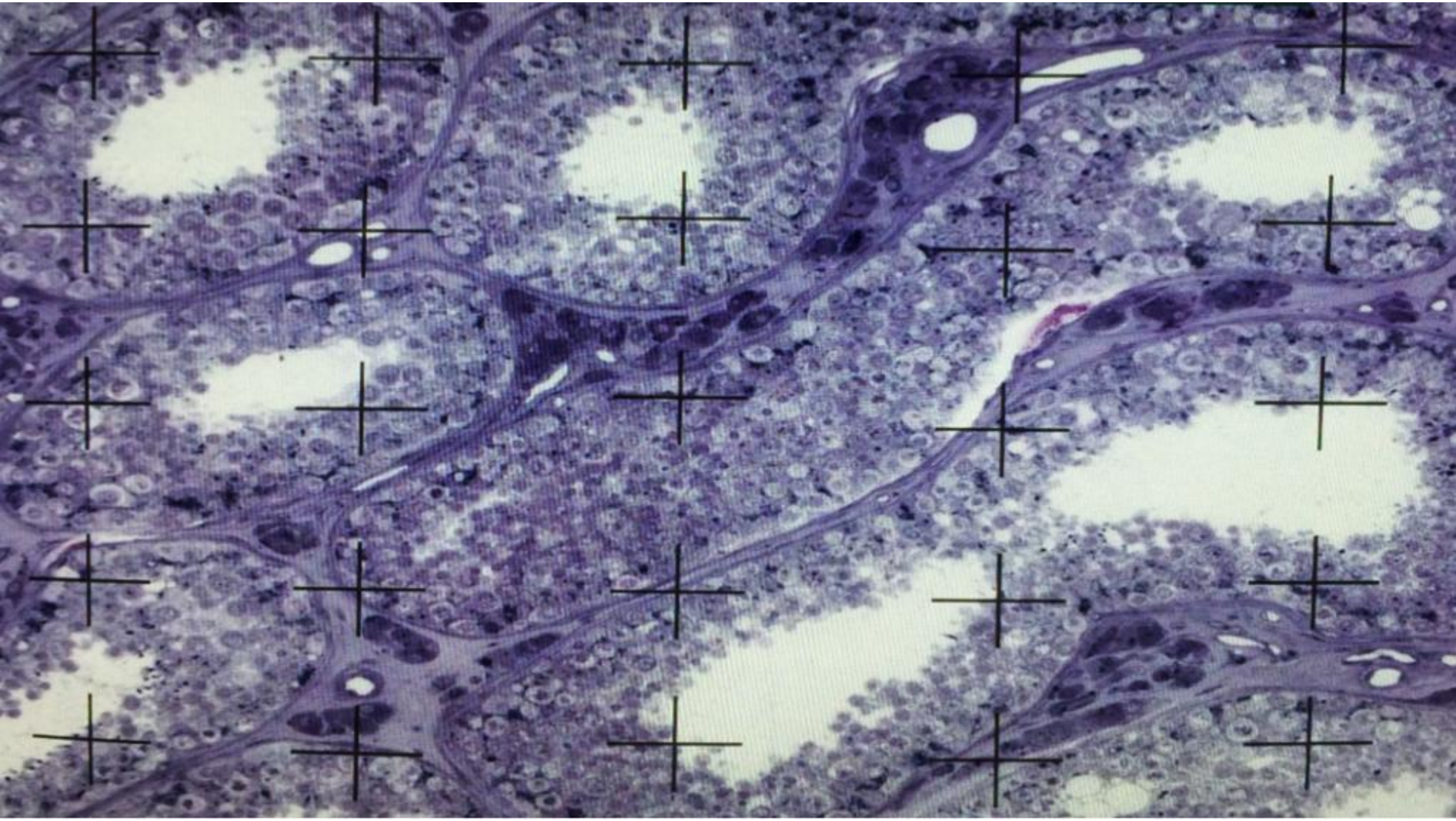


F. Record the numbers in the worksheet and add up totals.

G. Move to a new area and repeat hit count 4 or more times.

H. Add up the total hits that land on each structure from all repeated counts, divide by the number of possible "hits" (e.g., 5 repeats X 25 pluses per sheet = 125), and multiply that value by 100 to calculate tissue volume density.

Human Testes (number of hits on structures)
21 Seminiferous Tubules, 2 Boundary Tissue, 2 Leydig Cells, and 1 Blood Vessel



[Human Testes Image]

3. Tissue Volume Density Example Data

Seminiferous Tubules	Boundary Tissue	Leydig Cells	Blood Vessels
21	3	3	0
21	3	2	0
20	2	3	0
21	3	2	1
21	3	3	0

Total Hits

104	**14**	**13**	**1**

3. Tissue Volume Density Example Data:

percentage of whole occupied by each component

	Seminiferous Tubules	Boundary Tissue	Leydig Cells	Blood Vessels
Total Hits	**104**	**14**	**13**	**1**

Possible Hits 5 counts x 25 pluses per sheet = **125**

Volume Density of Tissue

$$\frac{104}{125} \times 100 = \boxed{\mathbf{83\%}}$$

$$\frac{14}{125} \times 100 = \boxed{\mathbf{11\%}}$$

$$\frac{13}{125} \times 100 = \boxed{\mathbf{10\%}}$$

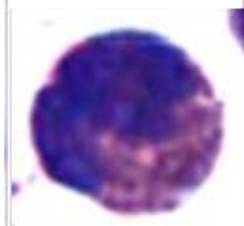
$$\frac{1}{125} \times 100 = \boxed{\mathbf{0.8\%}}$$

Conclusion: The bulk of volume of testicular tissue is occupied by seminiferous tubules, Leydig cells and tubular boundary tissue contribute about 10% each, and a low volume of blood vessels.

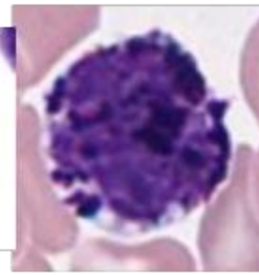
4. Percentages of Cells

Identify white blood cells

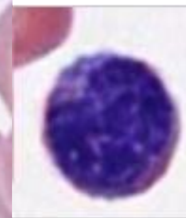
Neutrophil



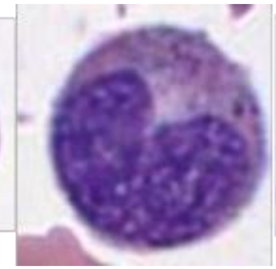
Eosinophil



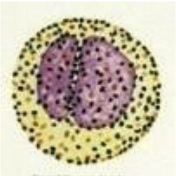
Basophil

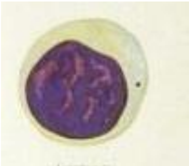


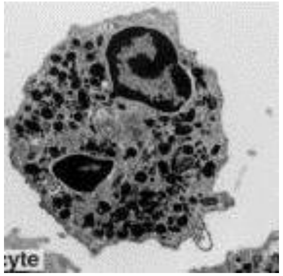
Lymphocyte



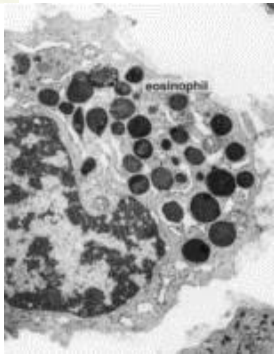
Monocyte



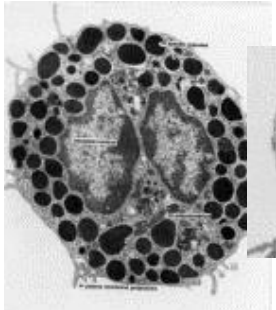




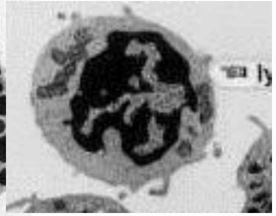
Neutrophil



Eosinophil

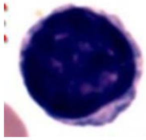


Basophil



Lymphocyte

Monocyte



Neutrophil

Eosinophil

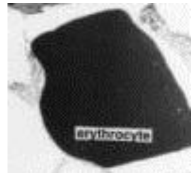
Basophil

Lymphocyte

Monocyte

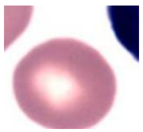
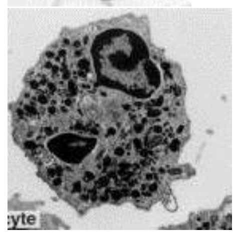
[Return to Toolkit]()

Human blood cells and functions

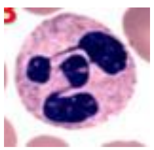
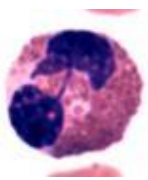
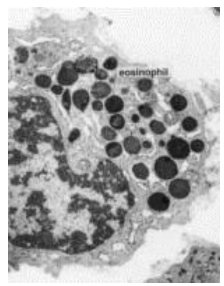


Cell type | **Main functions**

Erythrocyte — CO_2 and O_2 transport

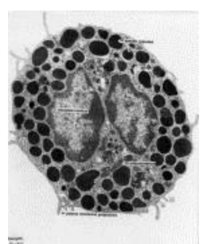



Neutrophil — phagocytosis of bacteria

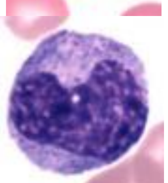




Eosinophil — parasitic infections, inflammatory processes

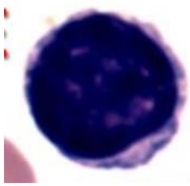
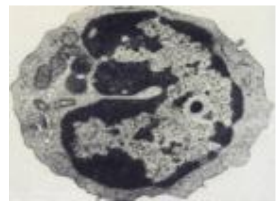
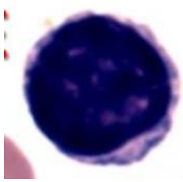
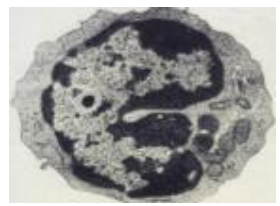
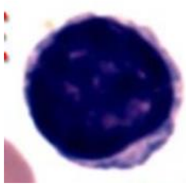
Basophil — release of histamine and other inflammation mediators

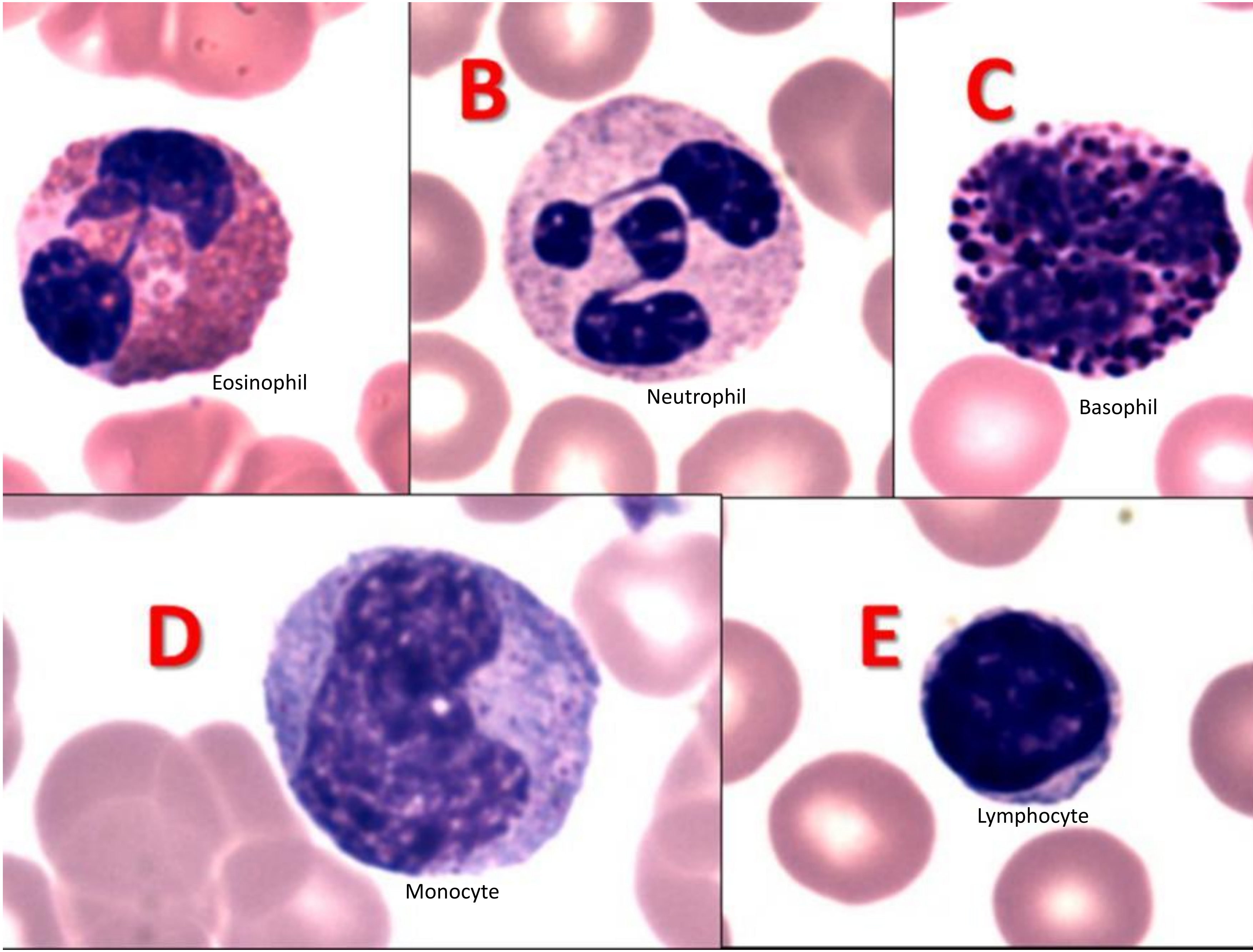



Monocyte — Mononuclear-phagocyte system become macrophages




Human blood cells and functions con’t

Cell type		main functions
 B lymphocytes		generation of antibody-producing plasma cells
 T lymphocytes		killing of virus-infected cells
 Natural killer (cytotoxic T cell)		killing of some tumor and virus-infected cells
 Platelets		clotting of blood


Eosinophil
B
Neutrophil
C
Basophil
D
Monocyte
E
Lymphocyte

4. Percentages of Cells

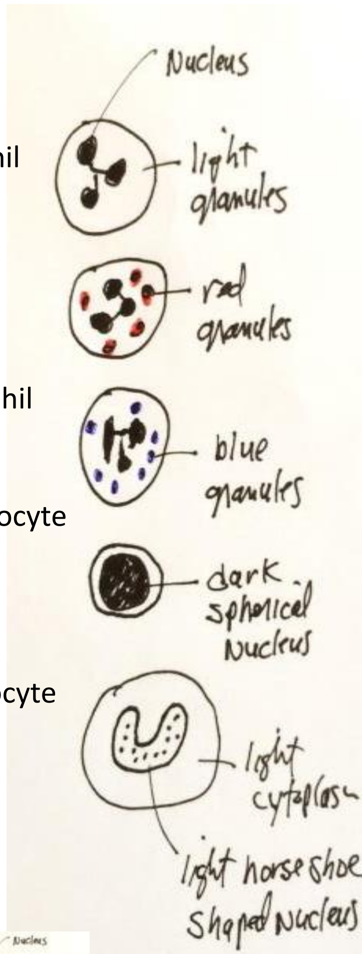
Neutrophil

Eosinophil

Basophil

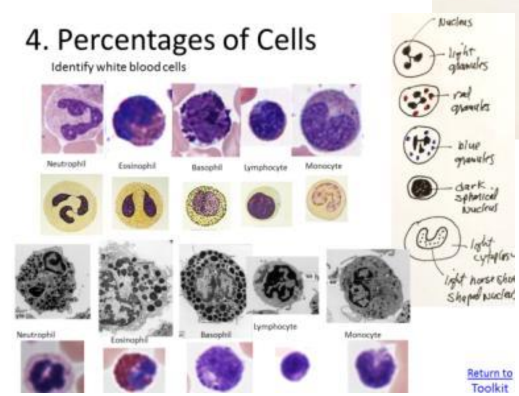
Lymphocyte

Monocyte



A. Open the healthy blood image, and set the magnification to 80x.
B. Pick at least 50 random white blood cells and classify which type it is based on images below.
C. Calculate the percentage of each WBC type found in the sample.

- Note, some WBC types are rare and you might not find any of them (e.g., basophils).



[Return to Toolkit]()

4. Percentages of Cells Example Data

Number of Cells in Healthy Blood

	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Counted	**46**	**8**	**6**	**2**	**1**

Total $46 + 8 + 6 + 2 + 1 = \mathbf{63}$

Percent

$\frac{46}{63} \times 100 =$ **73%**

$\frac{8}{63} \times 100 =$ **12%**

$\frac{6}{63} \times 100 =$ **9%**

$\frac{2}{63} \times 100 =$ **3%**

$\frac{1}{63} \times 100 =$ **1%**

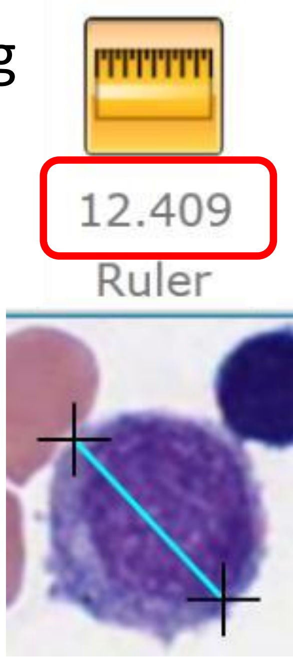
Conclusion : Neutrophils are the most numerous white blood cell in blood.

5. Diameter of Cells (Each white blood cell type)

A. Open the healthy blood image, and set the magnification to 80x.

B. Click on the ruler icon in the top toolbar to turn on ruler.

C. Place your arrow on one side of the cell, click and drag your pointer to the other side, and click to measure a cell's diameter.



D. Values are displayed beneath in micrometers (µm).

E. Record the values of 25 RBCs cells and 25 of each WBCs.

F. Calculate the average size of each cell.

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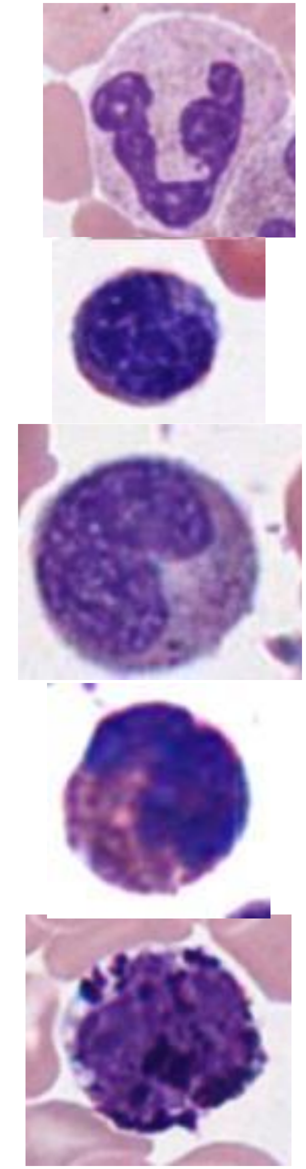
White blood cells



5. Diameter of Cells

- To turn off the ruler tool, click the ruler icon again.

- You must turn off the ruler to change magnification or move to a different area.

- Some white blood cells are rare, try to find and measure at least five eosinophils, basophils, and monocytes.

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5. Diameter of Cells Example Data

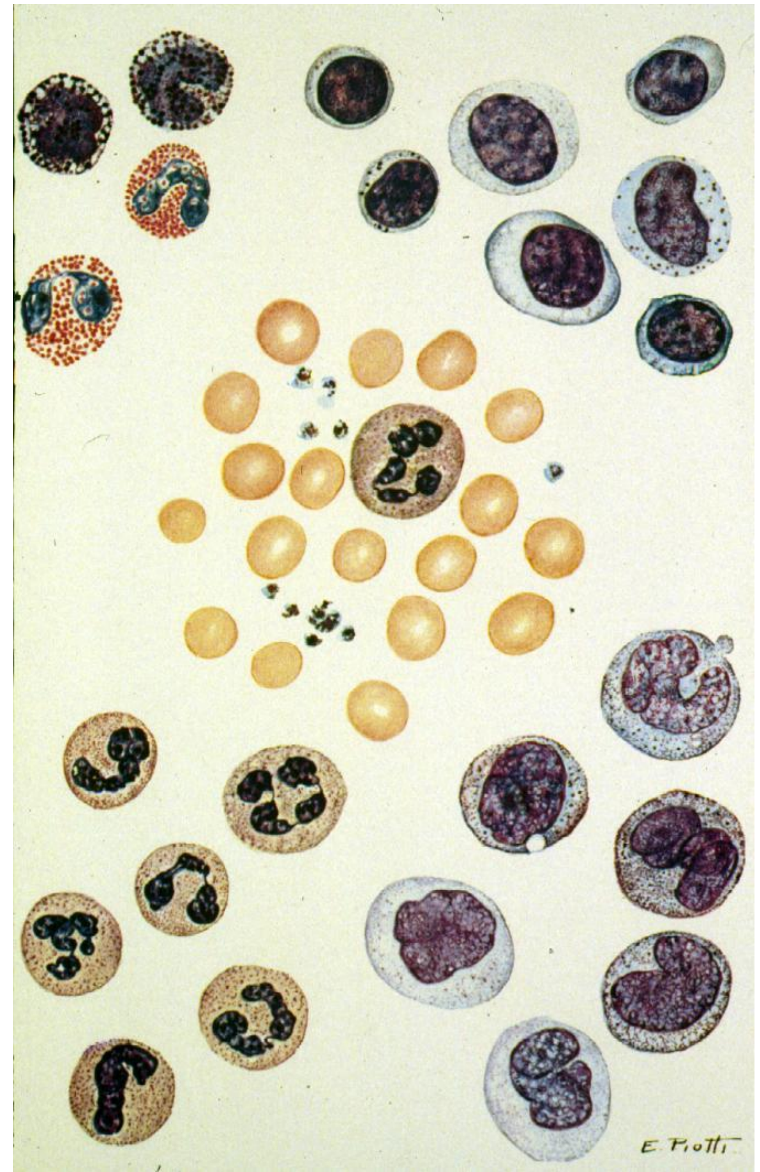
Healthy Blood Measured in micrometers (μm).

	RBC	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
	7.0	8.78	8.3	10.62	9.03	10.85
	7.5	9.76	7.94	10.64	10.1	9.71
	8.3	10.12	6.8	11.09	9.83	10.06
	7.1	9.89	6.99	11.65	10.49	7.95
	6.6	10.06	7.09	10.42	9.92	11.31
	…	…	…	…	…	…
	…	…	…	…	…	…
	7.1	8.58	6.85	12.59	9.25	7.89
Average Size	**7.36**	**9.38**	**7.31**	**10.51**	**9.64**	**9.63**

Conclusion: Blood cells vary in size (diameters) with the monocyte being largest with RBCs and lymphocytes being smallest.

Size of human blood cells

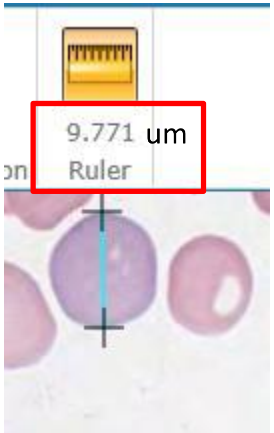
Cell/platelet	size
1. Erythrocytes	6.5-8 µm
2. Leukocytes (WBC)	
% of WBC	
a) Neutrophil	12-15 µm
60-70%	
b) Eosinophil	12-15 µm
2-4%	
c) Basophil	12-15 µm
0-1%	
d) Lymphocyte	6-18 µm
25%	
e) Monocyte	12-20 µm
5%	
3. Platelets	2-4 µm



5. Diameter of Cells (continued)

(Mature red blood cells and immature reticulocytes)

A. Open the unhealthy blood image, and set the magnification to 80x.
B. Click on the ruler icon in the top toolbar to turn on ruler.
C. Place your arrow on one side of the cell, click and drag your pointer to the other side, and click to measure a cell's diameter.
D. Values are displayed beneath in micrometers (µm).
E. Record the values of 25 or more RBCs cells and 25 or reticulocytes (blue, immature red blood cells).
F. Calculate the average size of each cell.



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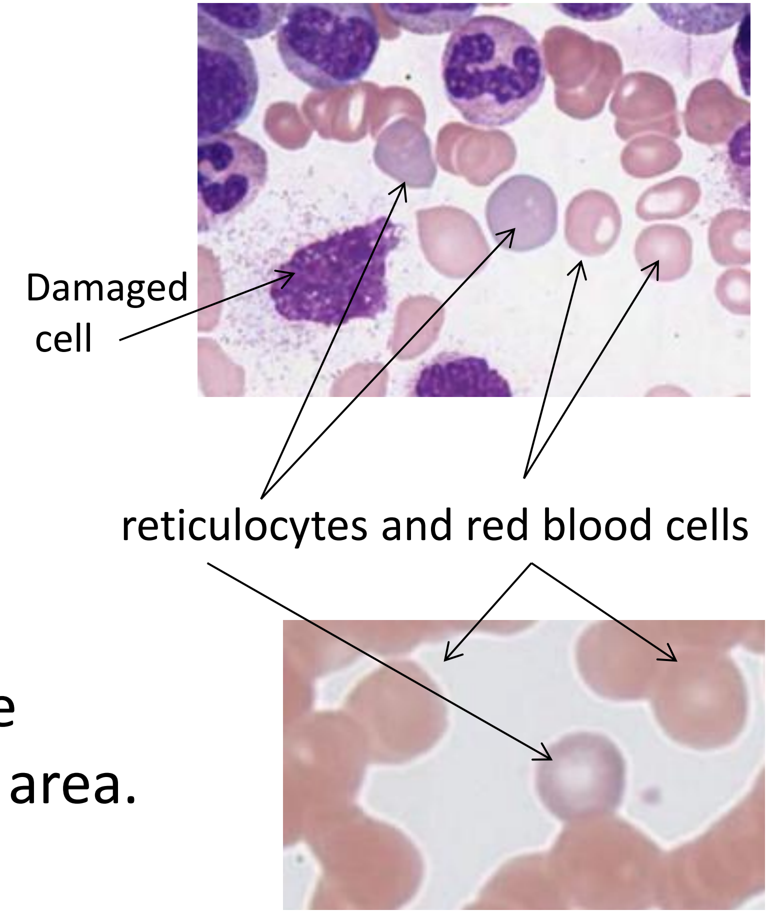
5. Diameter of Cells

- To turn off the ruler tool, click the ruler icon again.
- You must turn off the ruler to change magnification or move to a different area.
- It is more difficult to distinguish reticulocytes from red blood cells in the healthy specimen and the volume density is much lower in the health specimen. They may be too hard to find.

Unhealthy Blood



Healthy Blood

[Return to Toolkit]

5. Diameter of Cells Example Data

Measured in micrometers (µm).

	Healthy Blood		Unhealthy Blood	
	RBC	Reticulocyte	RBC	Reticulocyte
	7.24	7.66	7.0	8.66
	8.28	7.63	7.5	9.55
	7.95	8.02	8.3	7.9
	6.23	9.00	7.1	8.5
	6.20	7.96	6.63	8.31
	…	…	…	…
	…	…	…	…
	5.92	7.34	7.12	7.91
Average Size (µm)	6.97	7.93	7.36	8.46

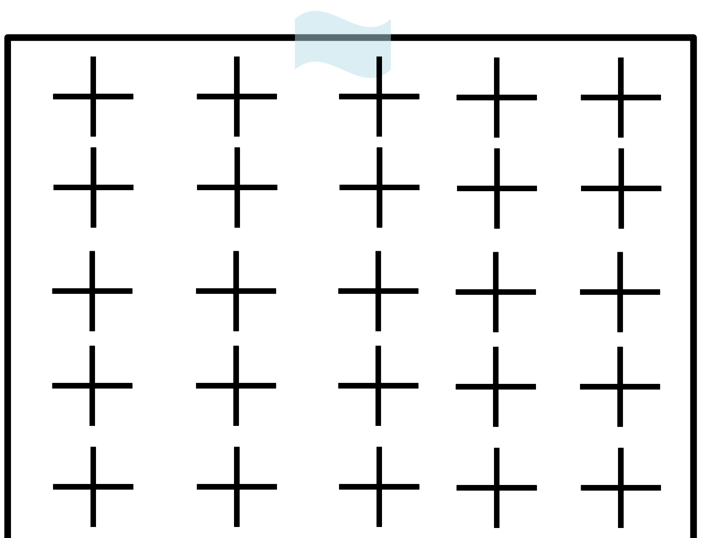
Conclusion: Reticulocytes are larger than RBCs for both the normal and abnormal blood samples. The reticulocytes were larger in unhealthy than healthy blood.

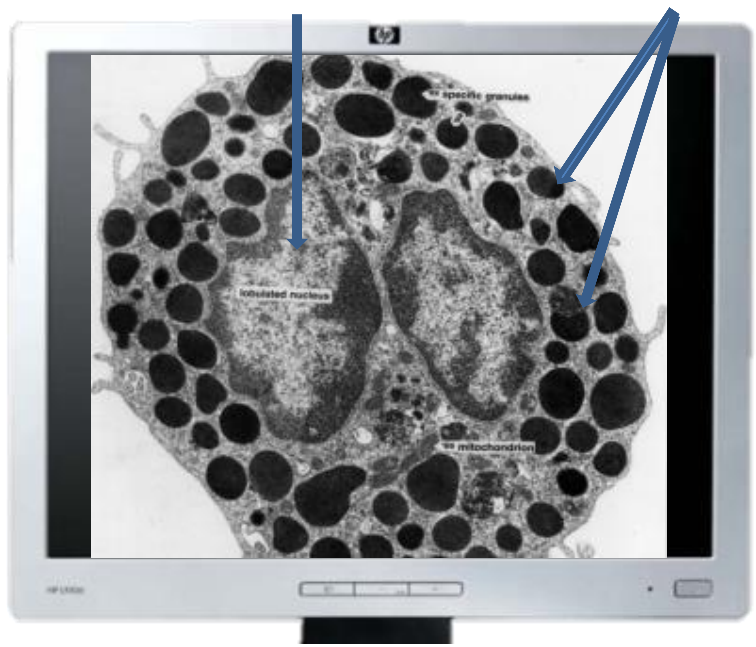
6. Organelle Volume Density

A. Print out the Plus Counter on a transparency sheet.

B. Open the electron micrograph to full screen.

C. Hold the Plus Counter transparency over the computer screen. Tape it in place if need be.





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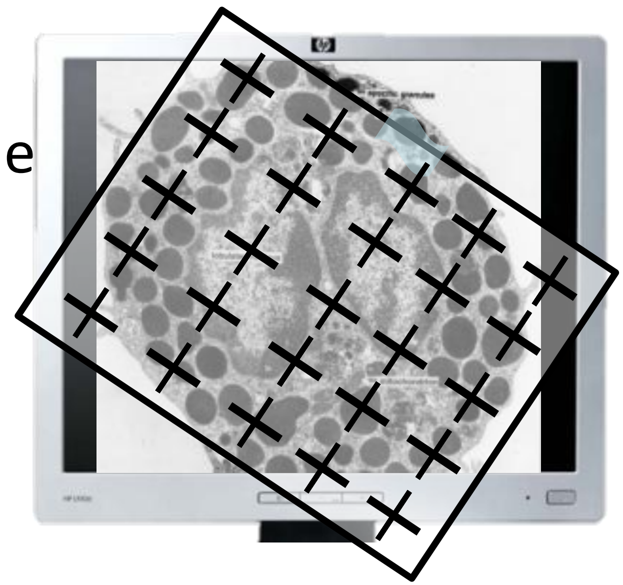
6. Organelle Volume Density

D. Count the number of organelles under the center of each cross (one "hit" is scored for each cross).

E. Count the number of crosses that touch nothing outside of the cell – this will be your possible hits total.

F. Rotate cross transparency 45 degrees to the right and recount. Rotate another 45 and repeat 1-3 more times.

G. Add up the total hits that land on each organelle from all repeated counts, divide by the number of possible "hits," and multiply that value by 100 to calculate volume density.



6. Organelle Volume Density – Example Data

Electron micrograph

Nucleus	Granules	Other	Total Possible
III	~~IIII~~ III	I	**12**
III	~~IIII~~	II	**10**
III	~~IIII~~ IIII	III	**15**

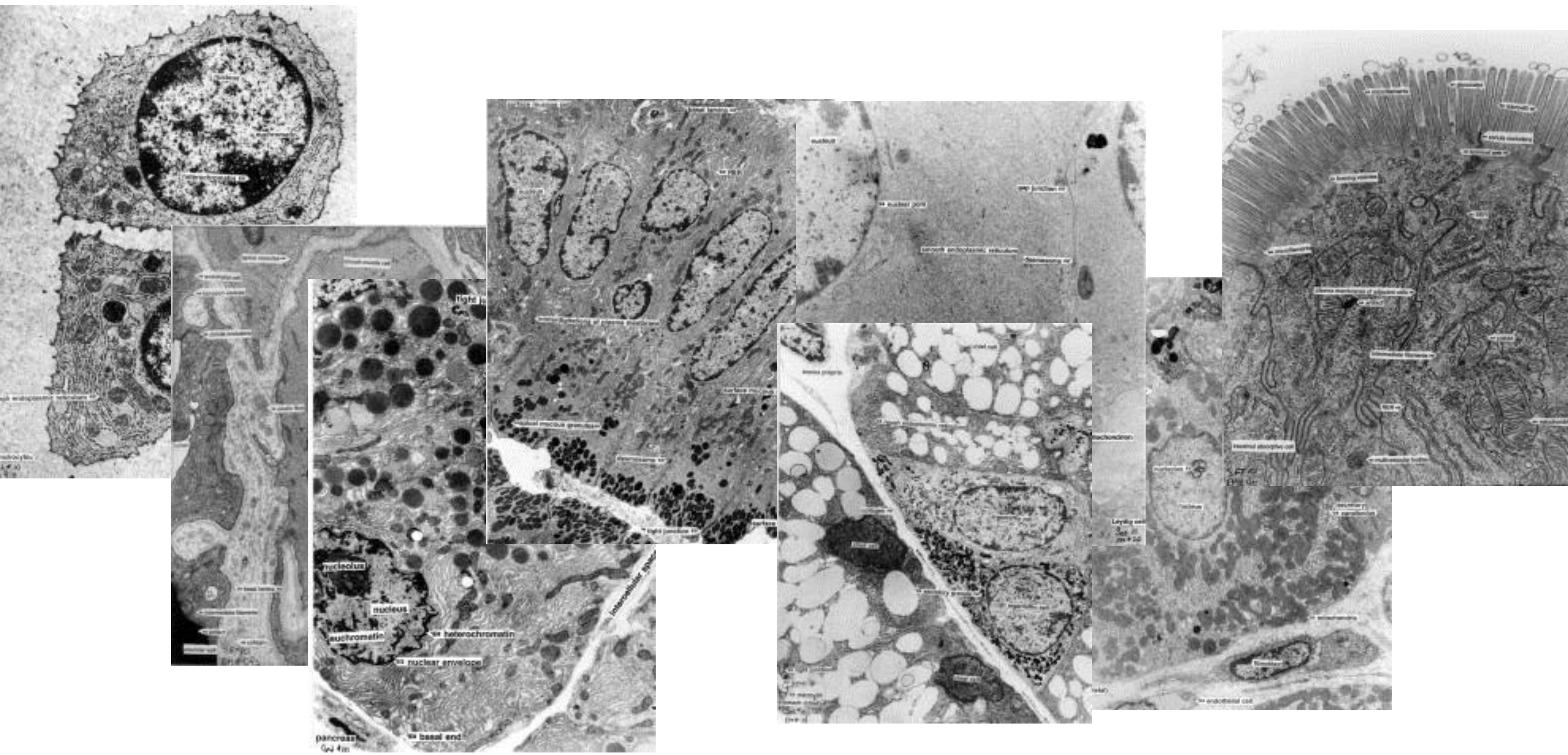
Total Hits	**9**	**22**	**6**	**37**

Organelle Volume Density

$\frac{9}{37} \times 100 = \boxed{\mathbf{24\%}}$ $\frac{22}{37} \times 100 = \boxed{\mathbf{59\%}}$ $\frac{6}{37} \times 100 = \boxed{\mathbf{16\%}}$

Conclusion: One forth of this cell was nucleus and 60% of this cell was granules.

Other electron micrographs that could be enlarged to fill the computer screen and be used to determine the volume density of organelles in different types of animal cells.



Toolkit Contents

VETERINARY MEDICINE & BIOMEDICAL SCIENCES
TEXAS A&M UNIVERSITY

Online Microscope Images Toolkit

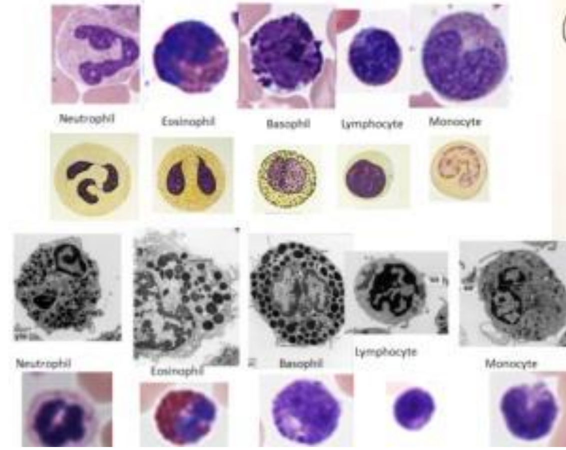
Larry Johnson, PhD

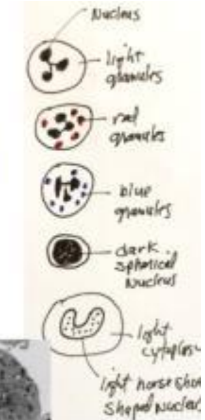
Partnership for Environmental Education and Rural Health (PEER)
peer.tamu.edu
YouTube: VIBS Histology

Veterinary Integrative Biosciences
Texas A & M University

4. Percentages of Cells

Identify white blood cells





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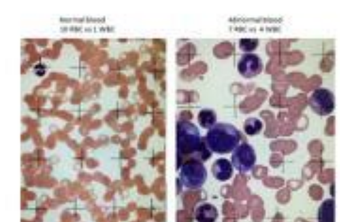
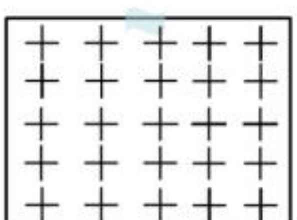
Why do cell counts (stereology)?

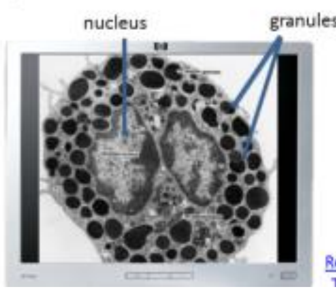
Stereology (random sampling of the whole group as opposed to counting all individuals in that group) allows quantitative analyses of images, cells, tissues, or micrographs to supplement general observations. It tells you how much change occurred not just that a change has occurred. This might indicate how sick a person is, how successful a treatment might be, or whether a person is getting better or worse. It might be used to determine if an observed change is statistically significant.



6. Organelle Volume Density

A. Print out the Plus Counter on a transparency sheet.
B. Open the electron micrograph to full screen.
C. Hold the Plus Counter transparency over the computer screen. Tape it in place if need be.





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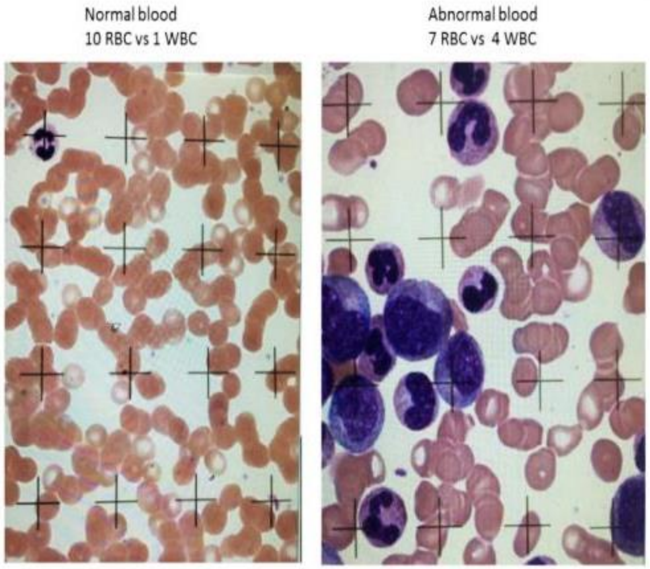
Conclusion

Stereology allows quantitative analyses of images, cells, tissues, or micrographs.

It might be used to determine if an observed change is statistically significant.



The end of

Online Microscope Images Toolkit

Larry Johnson, PhD

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YouTube: VIBS Histology

Veterinary Integrative Biosciences
Texas A & M University

YouTube video of toolkit
http://www.youtube.com/vibshistology

Link to transparencies

- http://peer.tamu.edu/drop_box/Cell%20counter%20Transparency.docx