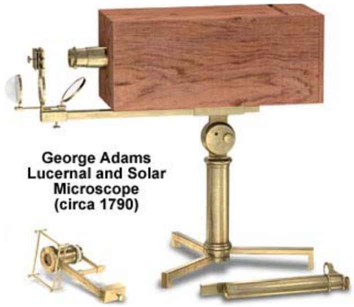
Cramer Brass Screw-Barrel Compound Microscope (circa mid 1700s)

Watson & Sons Compound Monocular Microscope (circa 1904)

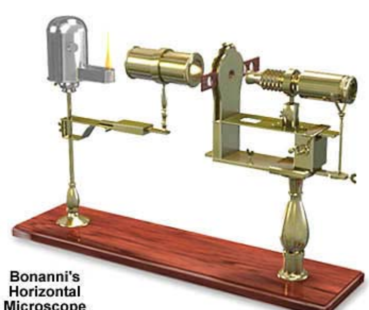


George Adams Lucernal and Solar Microscope (circa 1790)

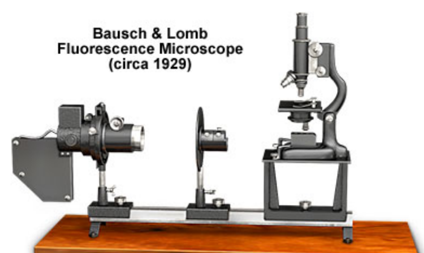
The Chest Microscope by Nairne & Blunt (circa 1780)

Kymograph

Leitz Photomicrographic Apparatus (circa 1910)



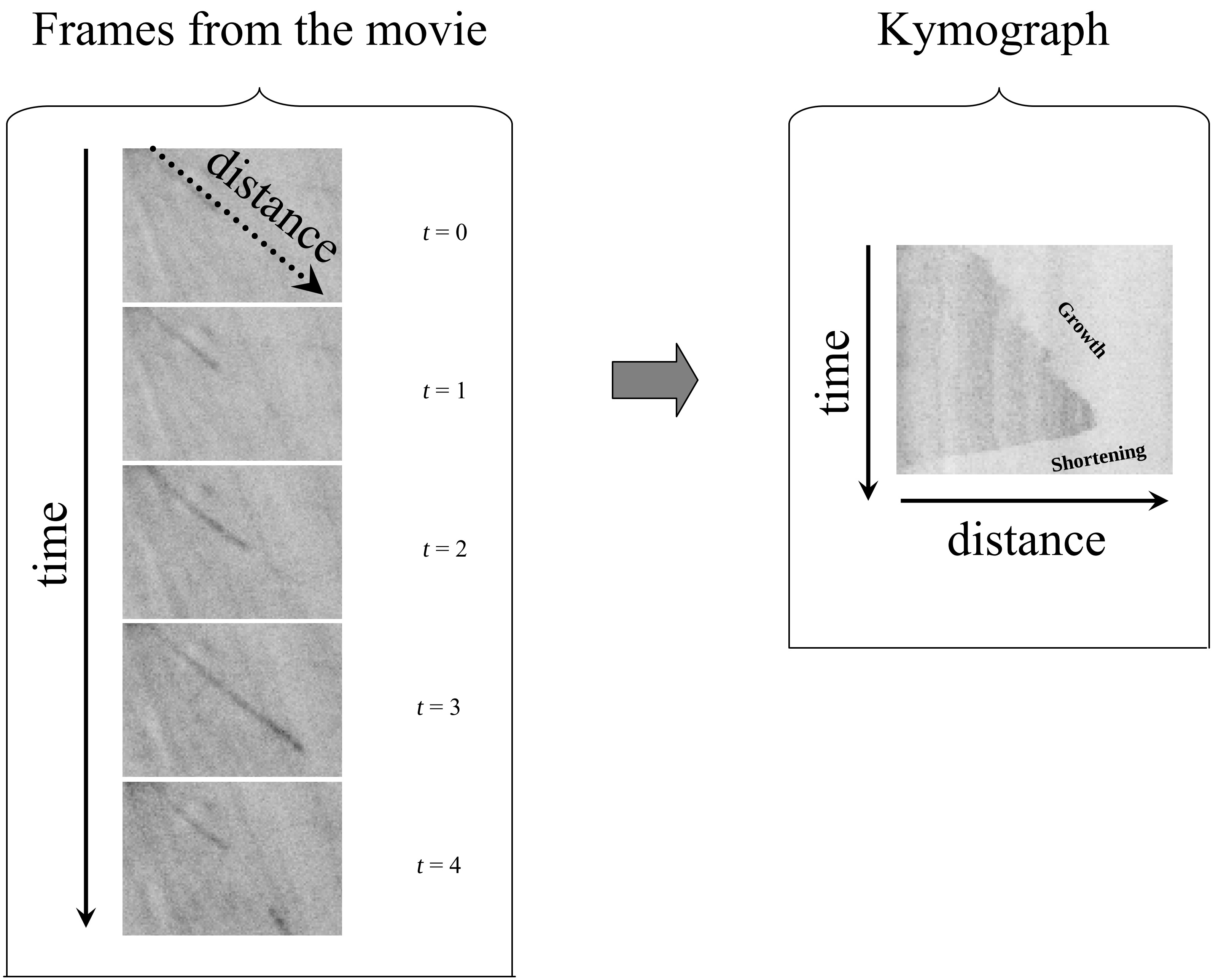
Bonanni's Horizontal Microscope (circa 1690s)



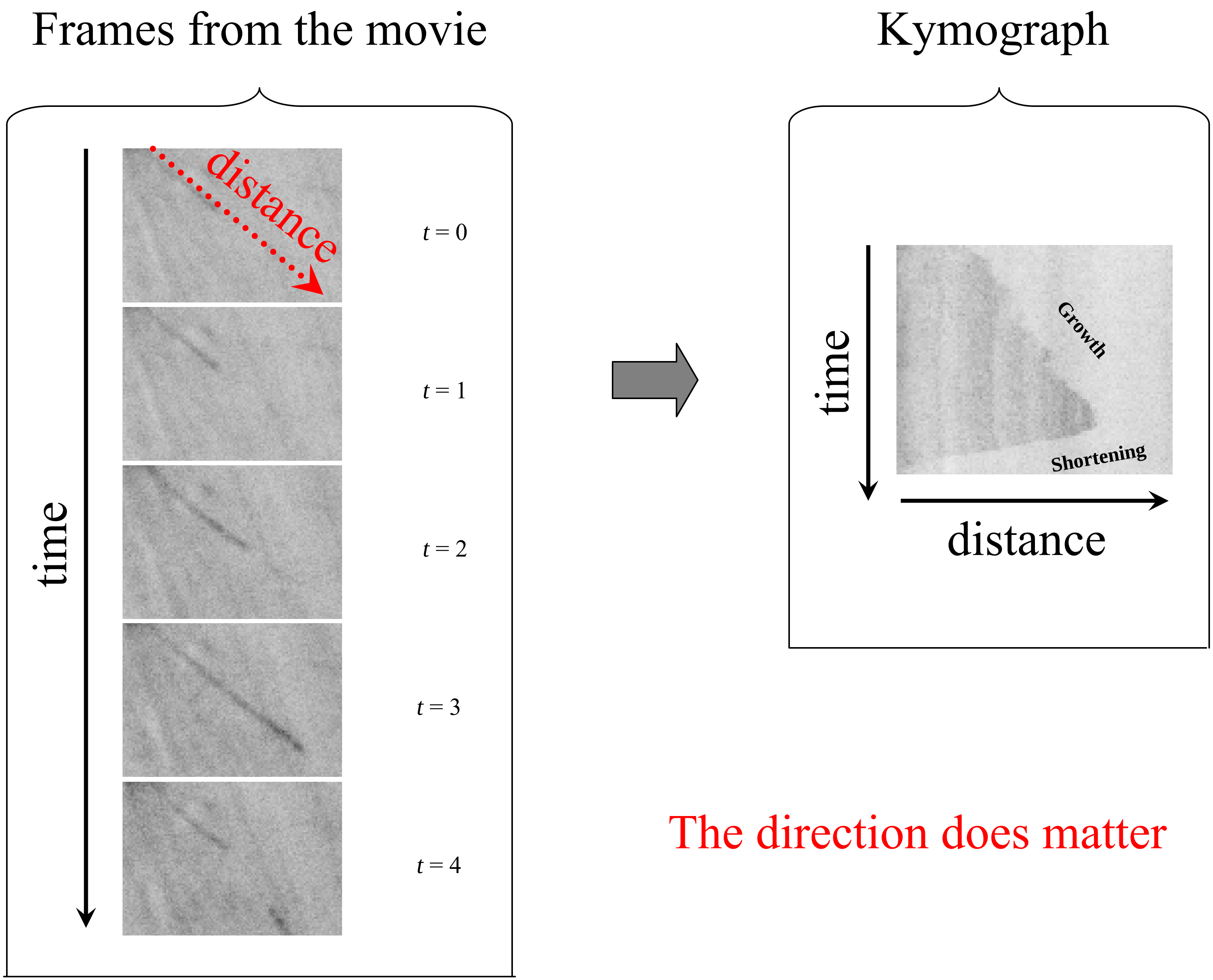
Bausch & Lomb Fluorescence Microscope (circa 1929)

The Chest Microscope by Nairne & Blunt (circa 1780)

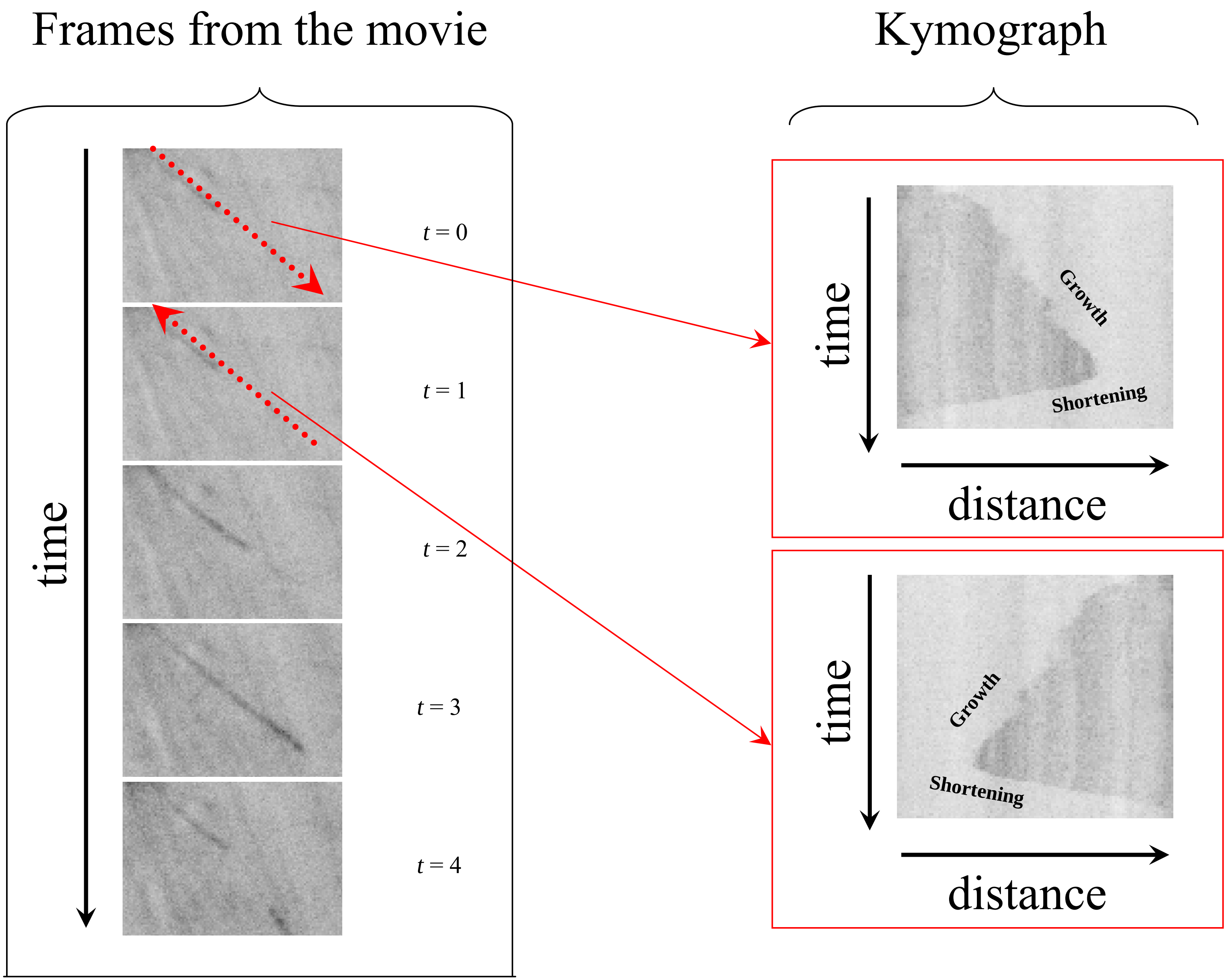
Kymograph graphically illustrates the changes in position over time



Frames from the movie
Kymograph
distance
time
t = 0
t = 1
t = 2
t = 3
t = 4
Growth
Shortening
time
distance

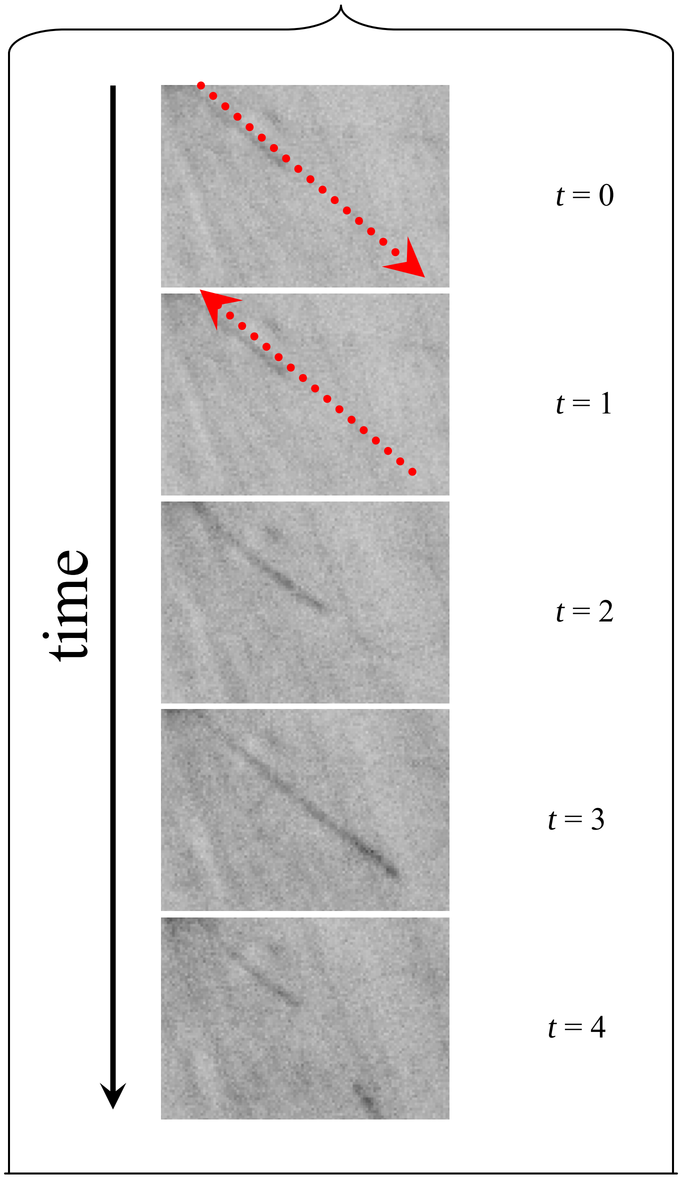


The direction does matter



Frames from the movie
Kymograph
time
t = 0
t = 1
t = 2
t = 3
t = 4
time
Growth
Shortening
distance
time
Growth
Shortening
distance

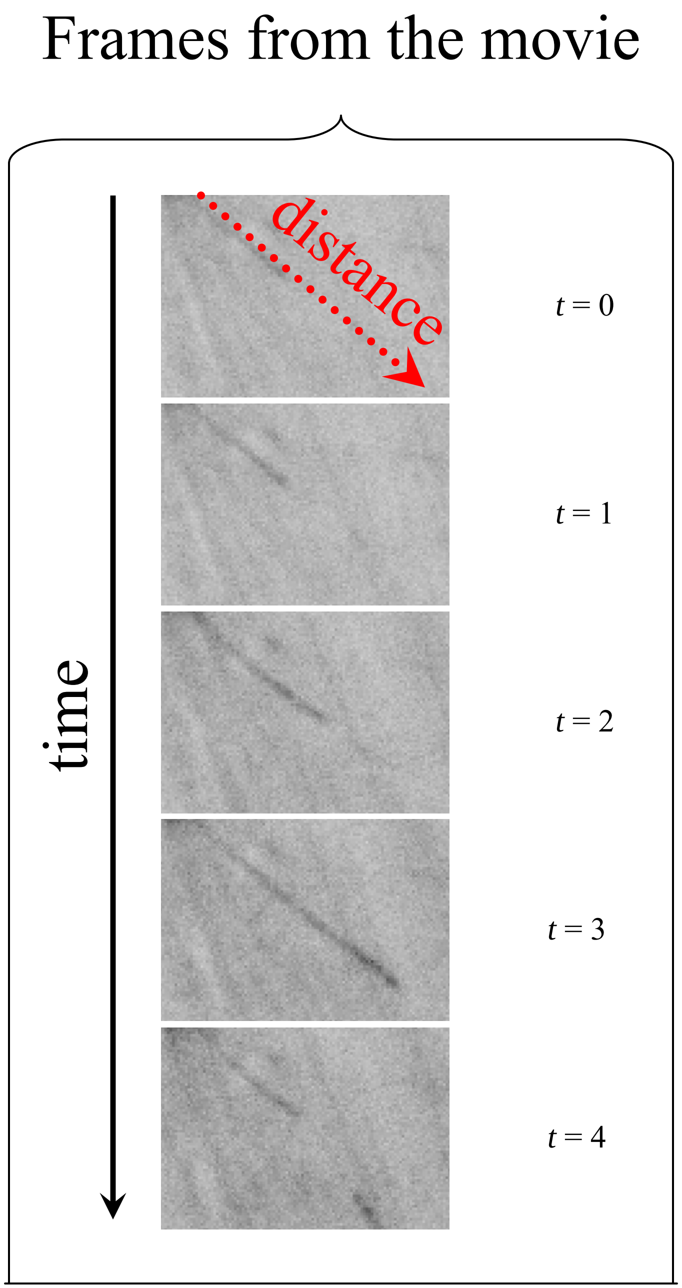
Frames from the movie

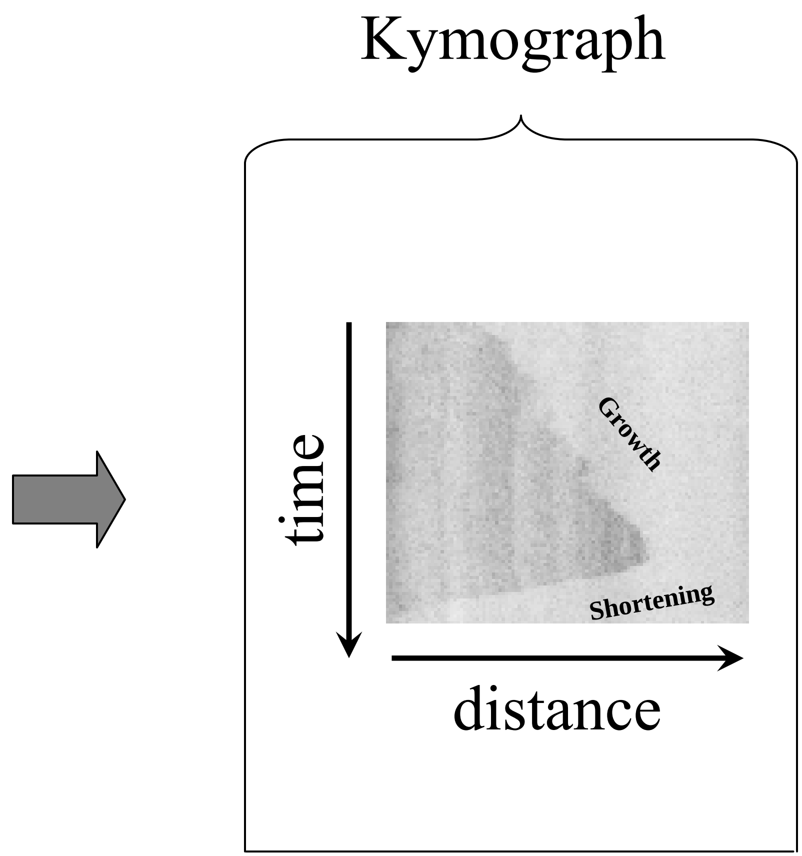


Which direction?

Does not matter.

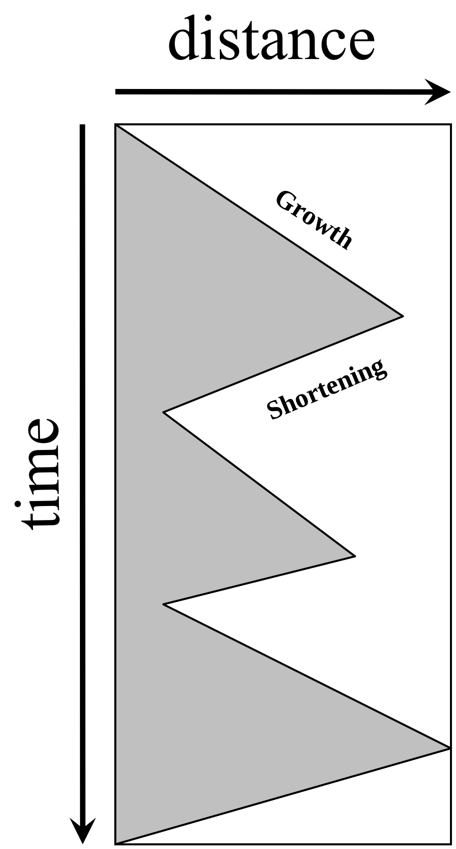
But you must be consistent
and draw the line
in the SAME direction
always!



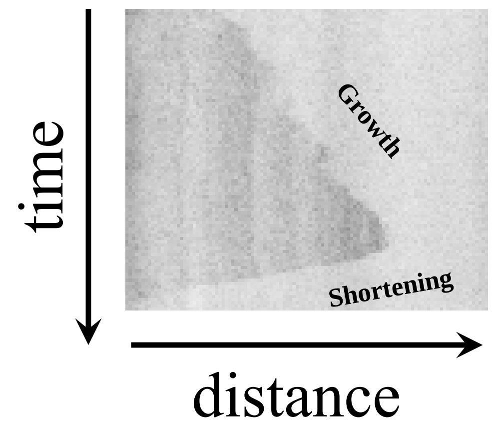


We chose this direction,
so all following explanations are
based on this scheme.

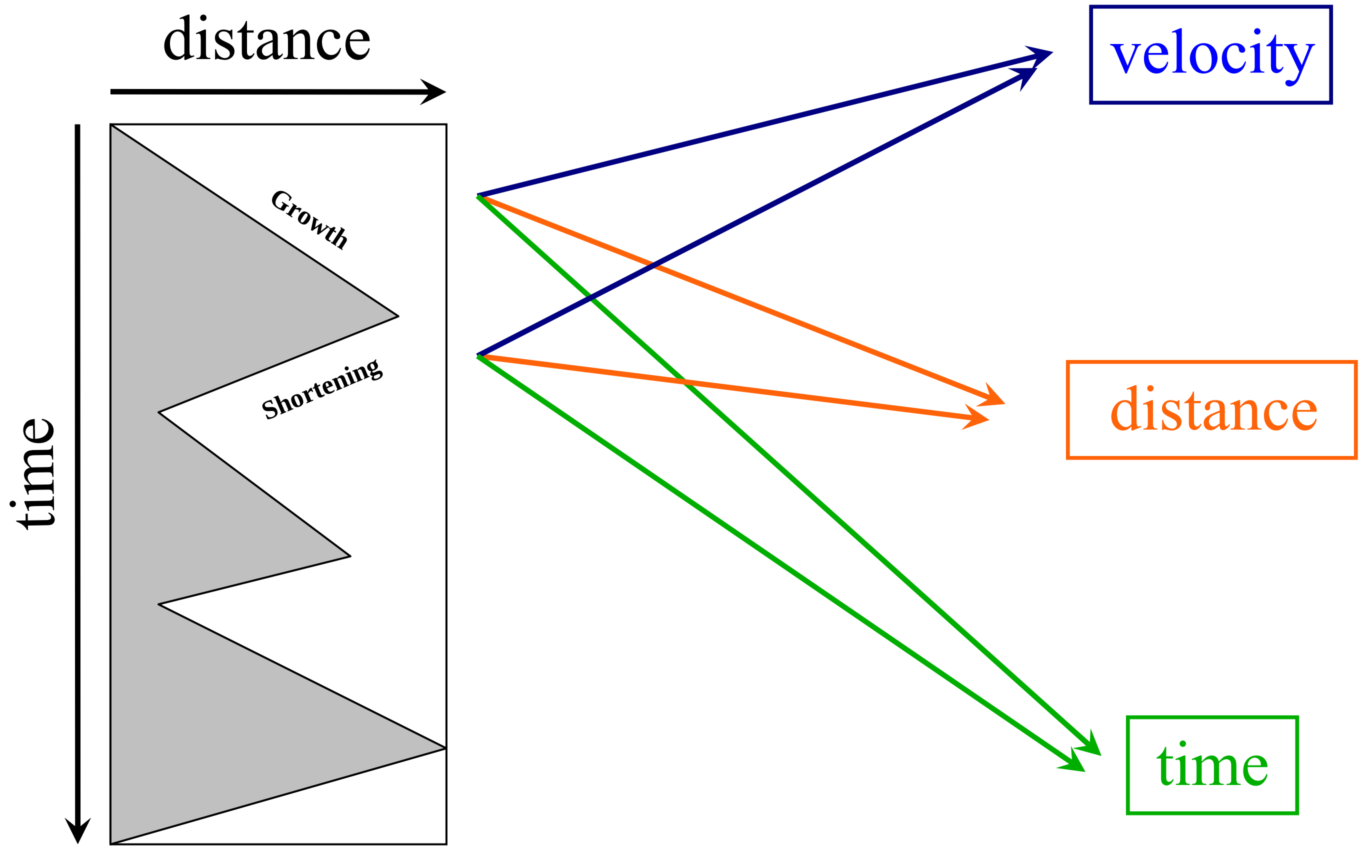
Kymograph analysis



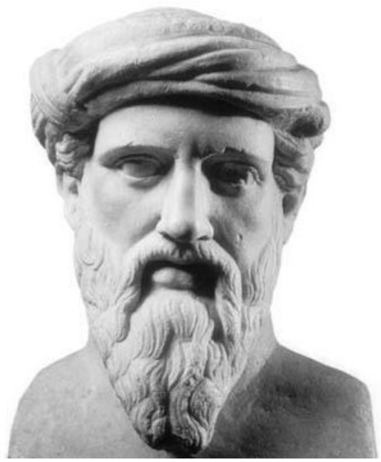
(schematic representation)



How to measure velocity, distance, time?



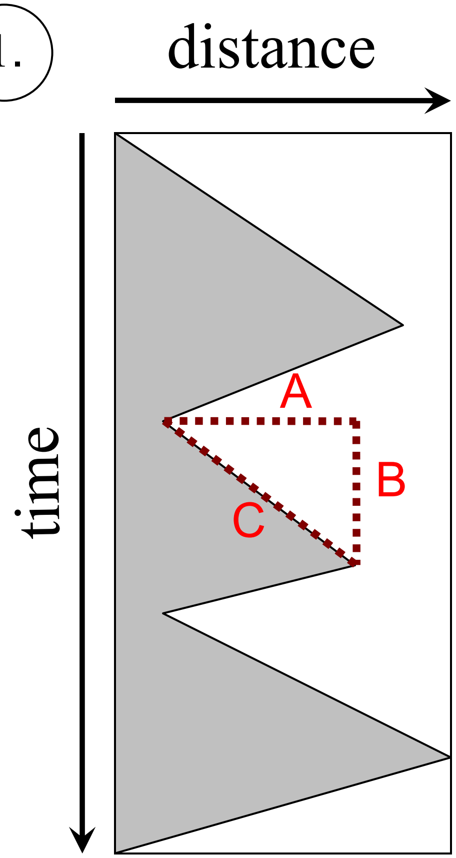
Some theory



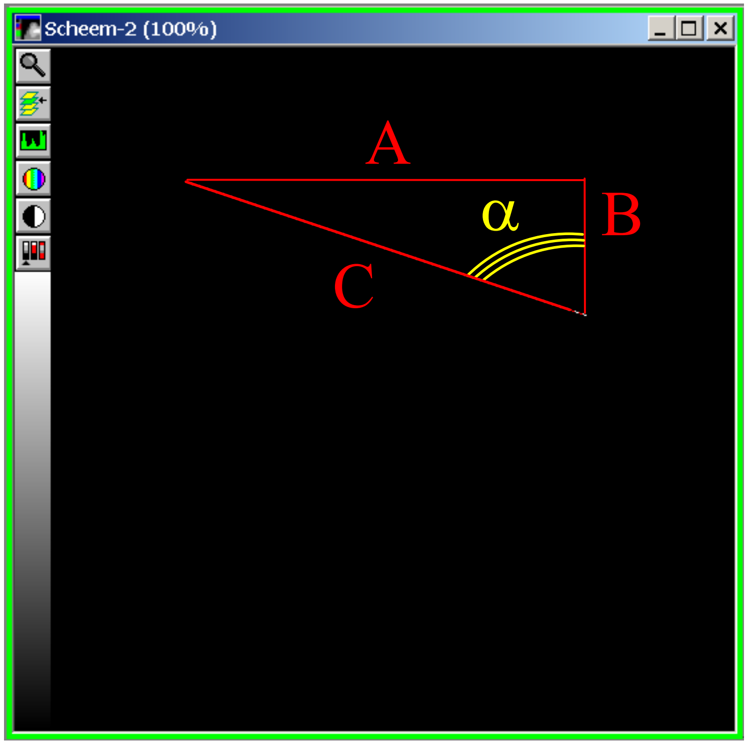
At school your maths teacher said that one day you would find a use for geometry or that algebra would save your life...

It's all in the angle action

1.

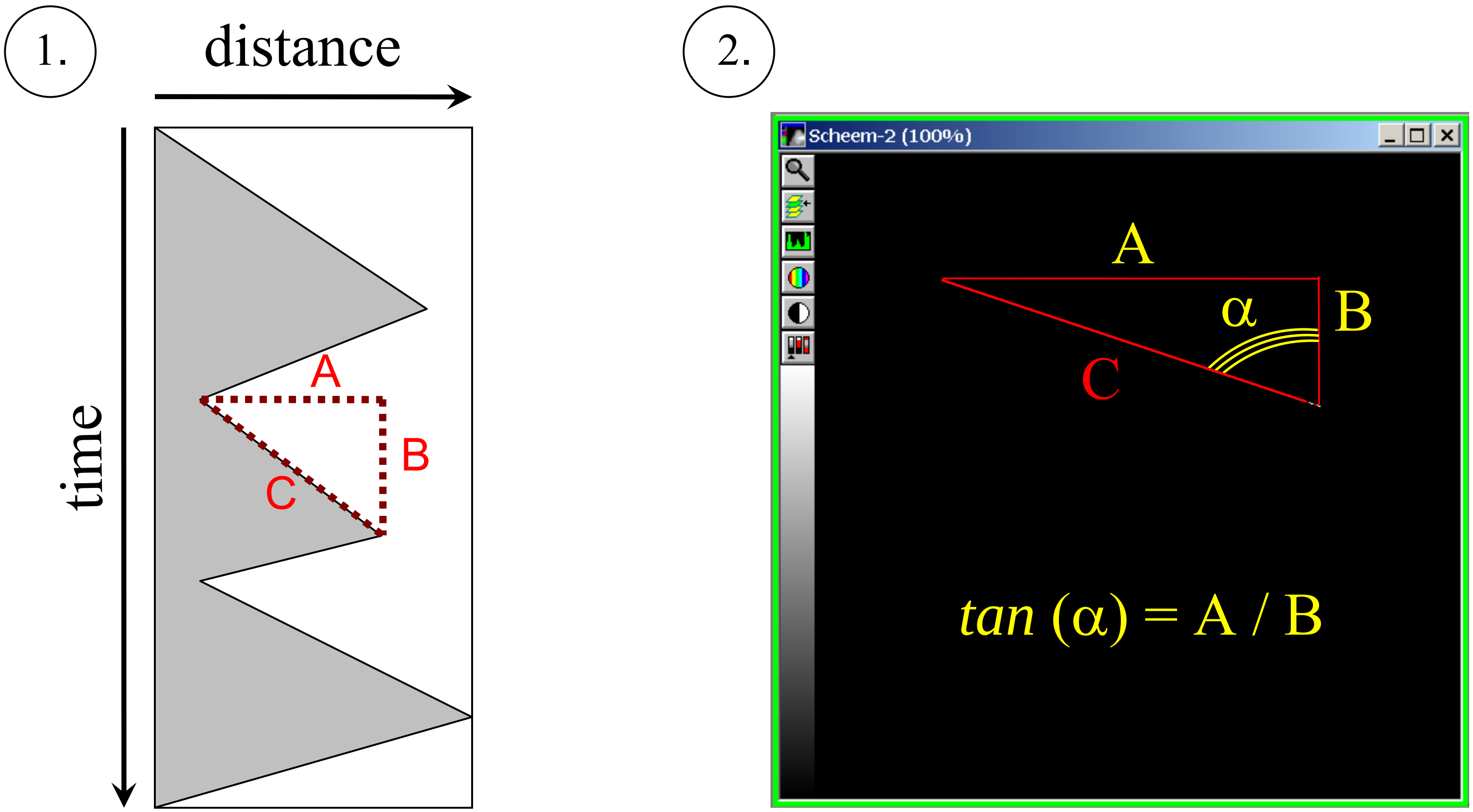


2.



In the cartoon kymo drawn in (1), the line C represents a growth phase.
If we make a right angled triangle as shown, then C is the hypotenuse.
We can make an angle between C and B... lets call it α, see (2).

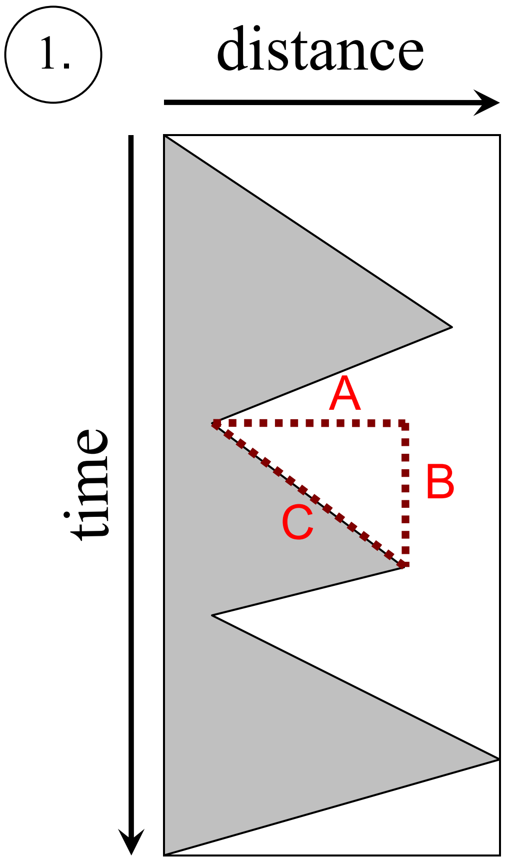
Velocity

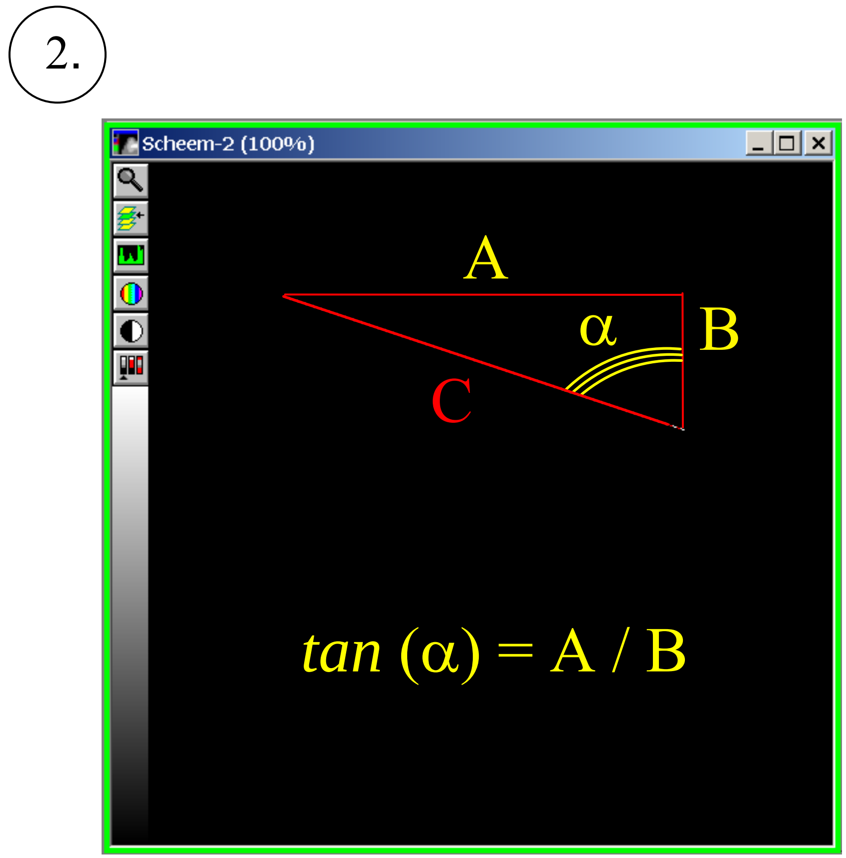


A is across from angle α and it known as the *opposite*.
B lies beside angle a and it known as the *adjacent*.
In a right angled triangle the tangent of an angle is:

tan = *opposite* / *adjacent* OR tan (α) = A / B

Velocity



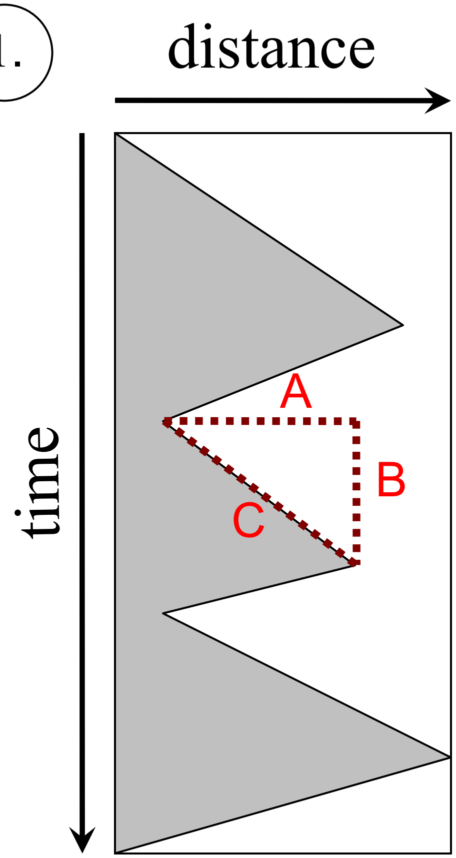


Since the *distance* is A and the *time* is B then
the velocity of growth must be A/B
To be correct the velocity is:

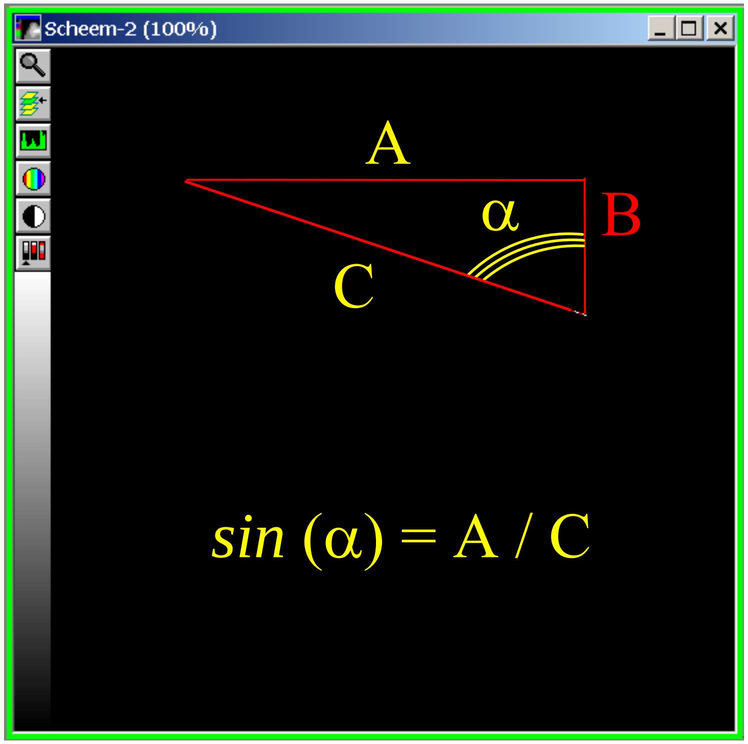
$$V = \tan(\alpha)$$

Distance...

1.



2.



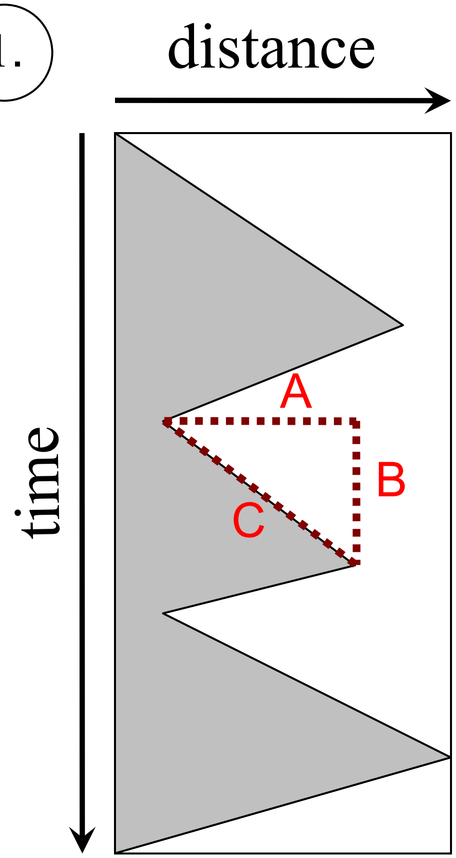
We can also work out the distance grown. In this case it would equal the length of line C multiplied by a factor dependent upon angle α.
Note, that in (1) distance is explicitly defined in the horizontal axis at 90°.

For the angle α the *sin* is equal to A / C. So the distance grown must be:

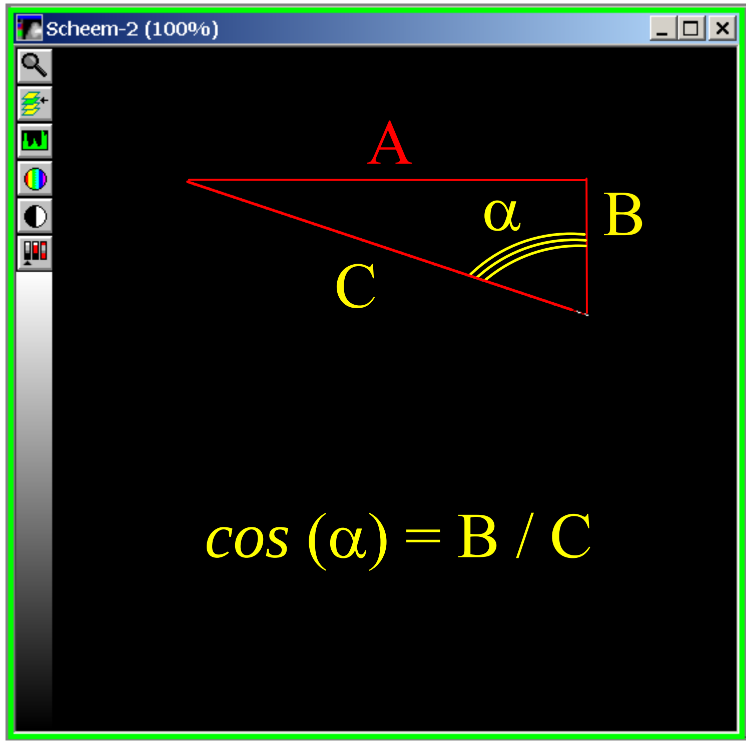
Distance = C * *sin* (α)

Time...

1.



2.

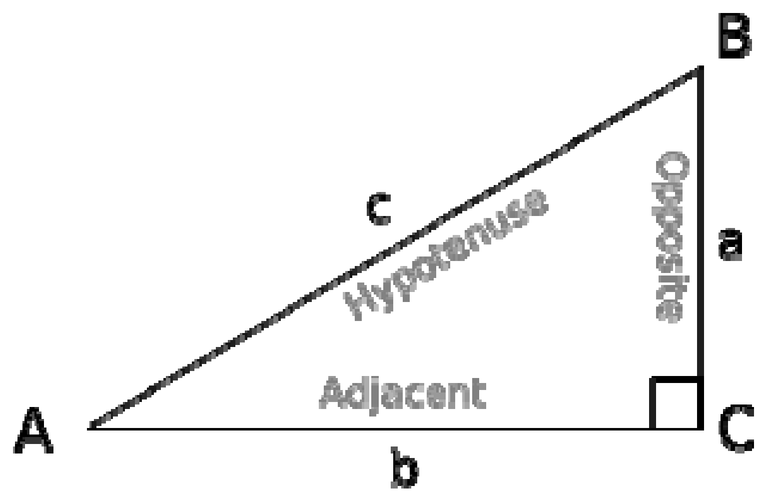


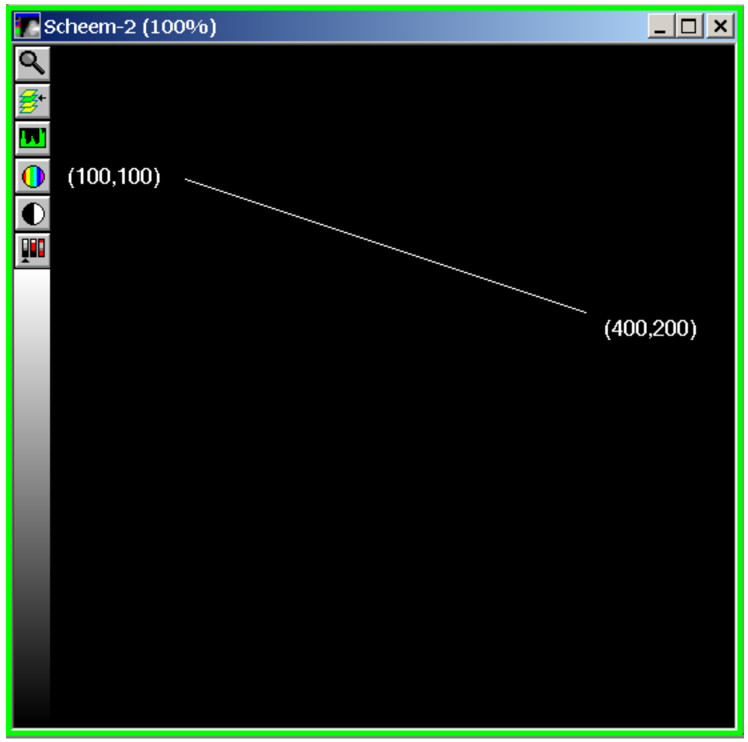
We can do the same thing for duration/time. In this case time would equal the length of line B multiplied by a factor dependent upon angle α.
Note, that in (1) time is explicitly defined in the vertical axis at 90°.

For the angle α the *cos* is equal to B / C. So the time or duration of growth must be...

Time = C * *cos* (α)

Practical example

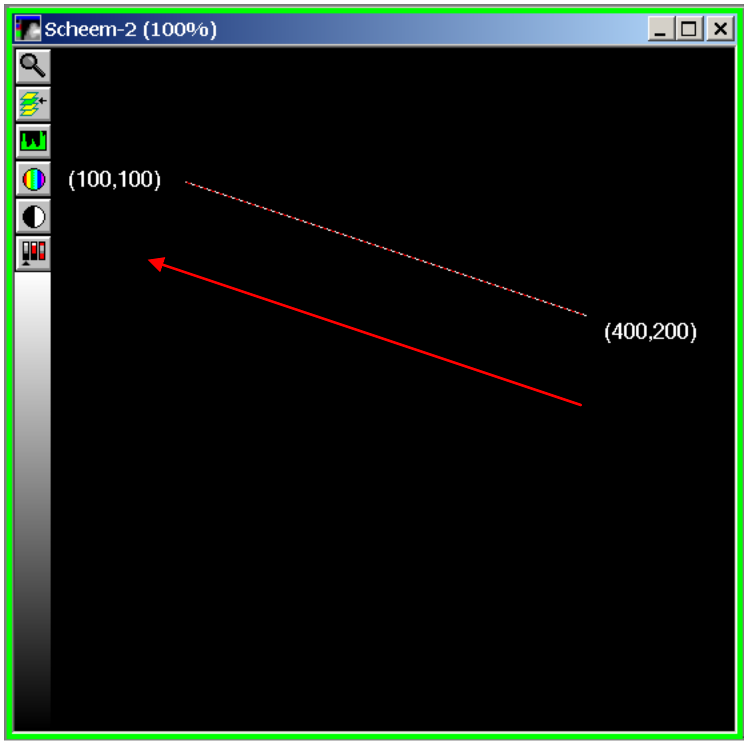




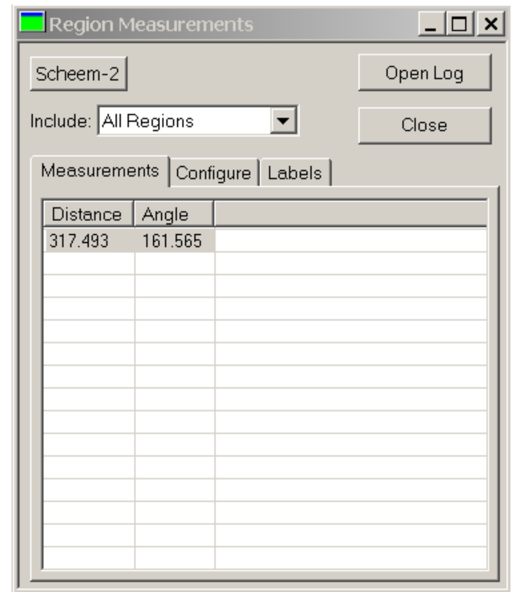
We would like to know velocity, distance and duration
of the movement happened along the white line in the figure above

Let’s draw the line above the segment (red short dashed line) in the direction shown with red arrow...

1.

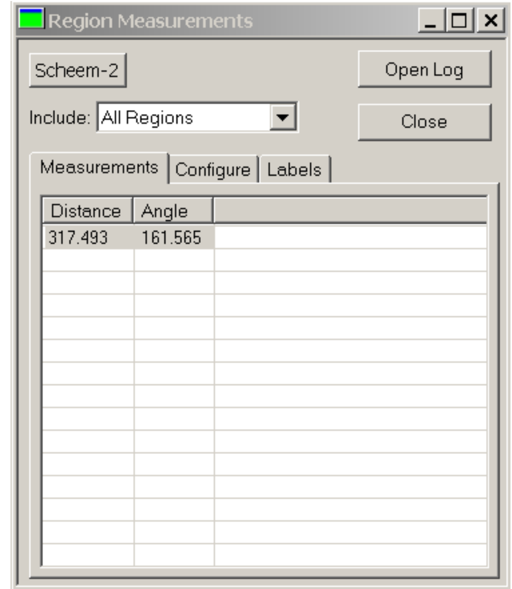


2.



MetaMorph calculates the distance and the angle for you. That's helpful but don't you think the value of that angle is a bit strange? Lets see why this is

MetaMorph has actually calculated the angle shown **NOT** the one we need!



Here's the calculation for velocity based on the given angle. In our case 15.4 pixels = 1µm and 2 frames = 1s of time:

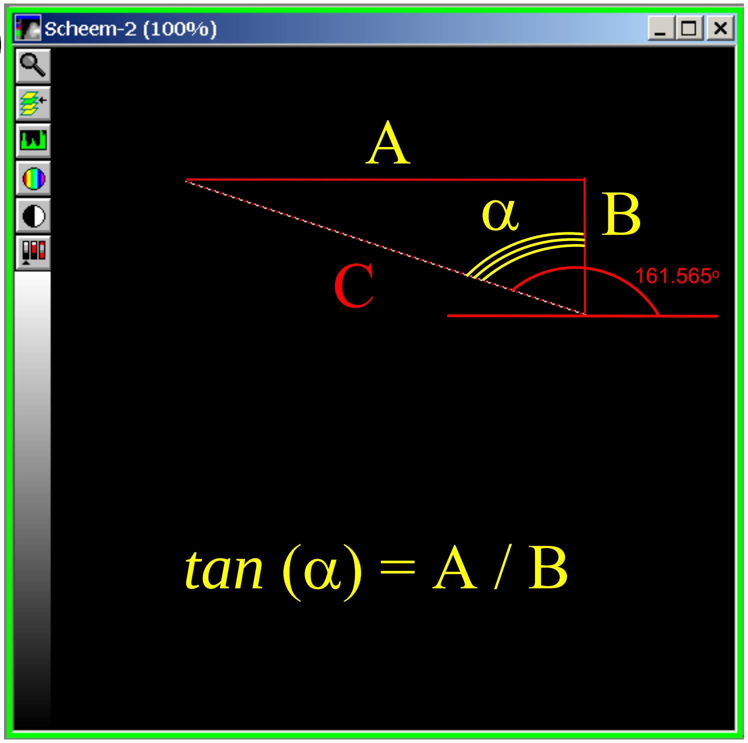
15.4 pixels = 1 µm
2 frames = 1 s

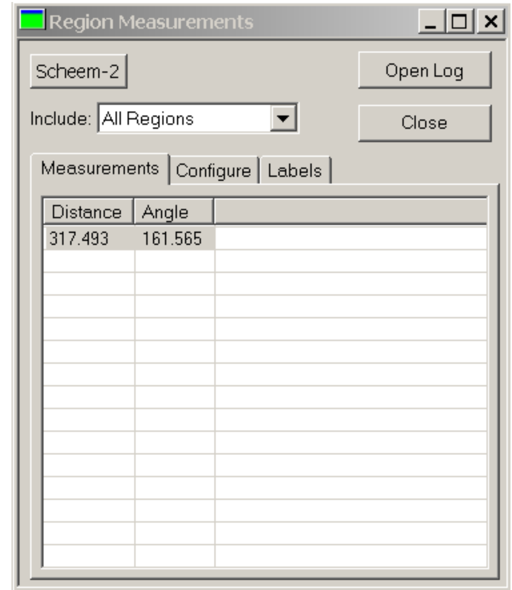
Velocity = tan (α) = tan (161.565º) = -0.33 pix/frame
Velocity = -0.33 * (-1) = 0.33 pix/frame *(a negative V is silly!)*
Velocity = 0.33 / 15.40 = 0.02 µm/frame
Velocity = 0.02 / 0.50 = 0.04 µm/s
Velocity = 0.04 * 60 = 2.40 µm/min

This is **wrong** and there will be tears before bedtime!

We can fix this and get the right angle... we just draw in our right angled triangle!

1.





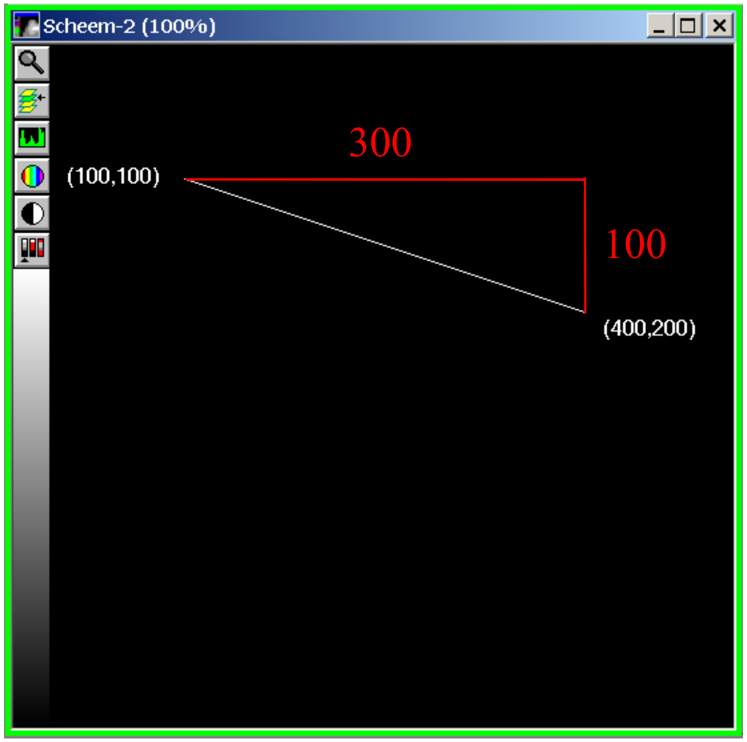
From (1) we can see that to get the correct angle (α) for the velocity calculation we need to subtract 90º from the one that MetaMorph gave us:

$$161.565^{o} - 90^{o} = 71.565^{o}$$

Velocity = tan (α) = tan (71.565) = 2.99 pix/frame
Velocity = 0.33 / 15.40 = 0.19 µm/frame
Velocity = 0.02 / 0.50 = 0.39 µm/s
Velocity = 0.04 * 60 = 23.37 µm/min

Does this make any sense?

We can validate it by knowing the line length in pixels of each side of the triangle.



Velocity = 300 pixels for 100 frames

(NB: v = d / t)

Velocity = 300/100 pixels/frame

Velocity = 3.00 pixels/frame

15.4 pixels = 1 μm

2 frames = 1 s

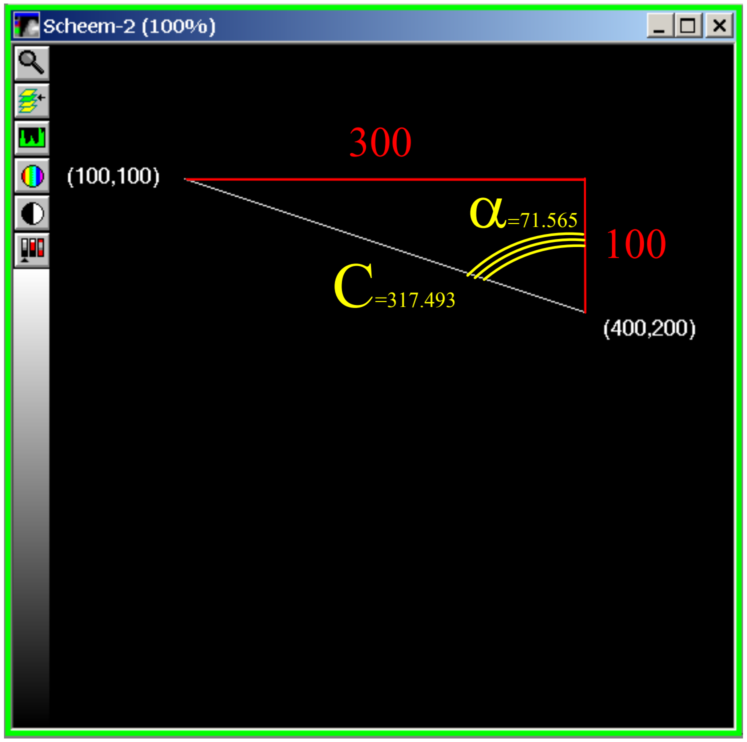
Velocity = 3.00 / 15.40 = 0.19 μm/frame

Velocity = 0.19 / 0.50 = 0.39 μm/s

Velocity = 0.39 * 60 = 23.37 μm/min

All small deviations are based on pixel errors / measurement error.

With the line lengths we can now work out the distance...



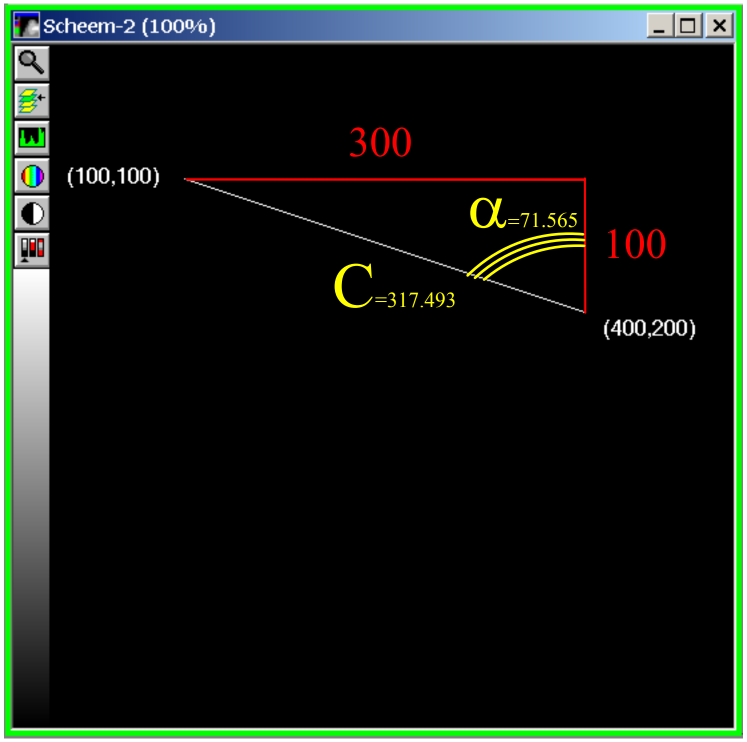
Distance = C * *sin* (α)

Distance = 317.493 * sin(71.565) pixels

Distance = 301.2 pixels

All small deviations are based on pixel errors / measurement error.

...and the duration.

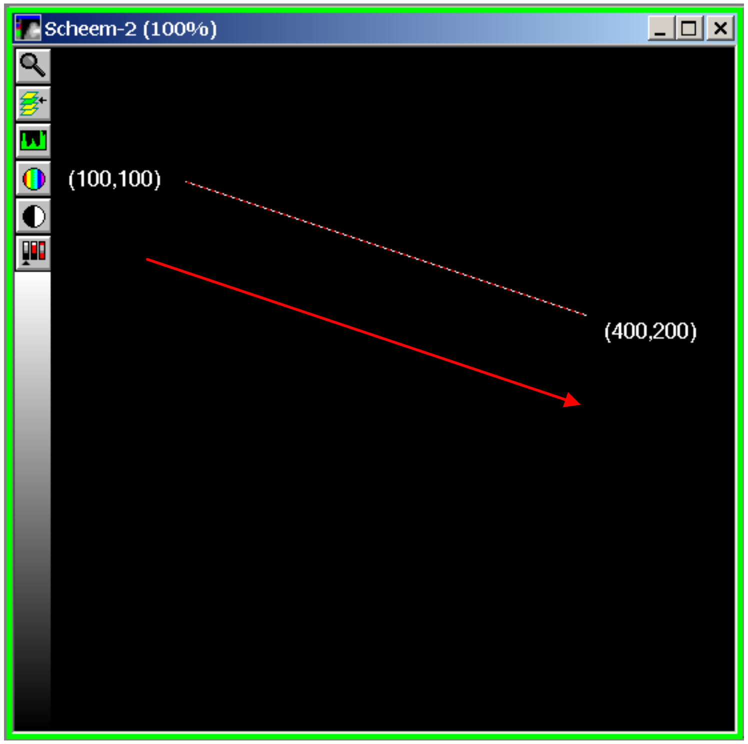


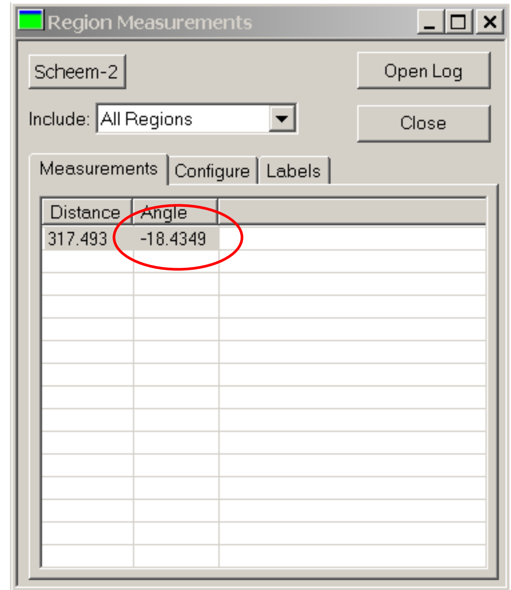
Duration = C * *cos* (α)

Duration = 317.493 * cos(71.565) pixels

Duration = 100.4 pixels

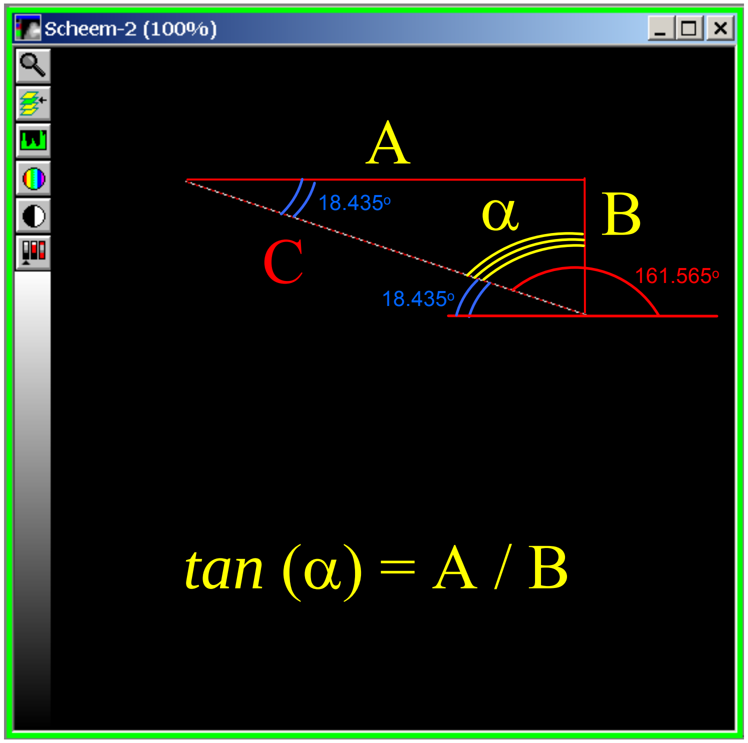
At the beginning we said: “be consistent”! Here's why...

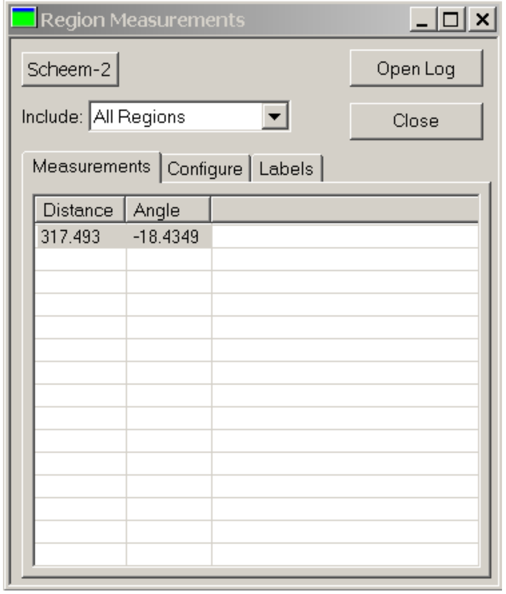




IF you draw the line in the OPPOSITE direction (shown with red arrow) MetaMorph calculates the distance and ANOTHER angle! Be careful!

Even if you choose to ignore our advice you can still fix things





The correct angle (α) is given by:

$$180^{o} - 18.435^{o} - 90^{o} = 71.565^{o}$$

Info:

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