

Lights, Camera, Action!

Creating and using image stacks



Lesson

Have you ever wanted to make a movie? Well, here's your chance! With your imagination and a little help from image processing technology you can easily create digital animations using stacks of images. You can even save them as QuickTime® and AVI movies for use with other applications.

At the movies

First, let's observe a ready-made animation.

- Open **Galloping Horse.tif**.
- Drag the scroll bar at the bottom of the stack window left and right to see the frames, or *slices*, of the stack.

This series of images was created in 1878 by Eadward Muybridge, an early pioneer in the use of photography to study animal locomotion.

- Press the backslash (\) key while holding down **Control** (Win) or **Command** (Macs) to start the animation. Click on the image to stop the animation, or use the \ key alone.
- Experiment with changing the speed of the animation. To change the speed, choose **Image > Stacks > Animation Options....**

1. What are the fastest and slowest animation speeds in frames per second?

What is a stack?

A set of images displayed within a single window is called a *stack*. A stack is similar to a pad of paper or a deck of cards, except that each "card" is a digital image. These images can show many things—a sequence of events, parts of a larger image, or slices through an object at different depths. In fact, ImageJ refers to the individual, "still" images within the stack as *slices*.

This lesson will focus on the use of stacks for animating a series of images. Perhaps you have created a simple flip-book animation using a stack of paper. When the images are shown quickly, the eye "sees" motion. This is the basic principle that is used for making movies. Films and videos are really just series of still pictures shown in a rapid sequence. By arranging a series of images in a stack and flipping through them, you can create your own animation.

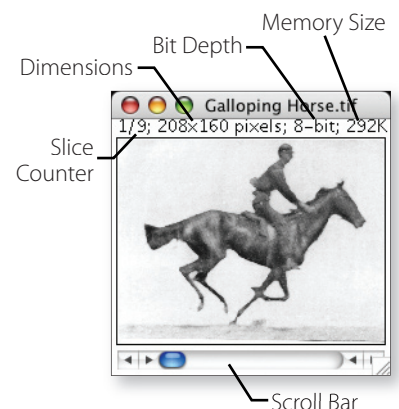
- Use the > and < keys or the scroll bar to move forward and backward through the stack as you view the individual slices.
- Below the title bar of the **Galloping Horse.tif** window is a row of numbers. The first number refers to the slice of the stack that you are currently viewing, and the second number refers to the total number of slices in the stack.

2. How many slices are there in **Galloping Horse.tif**?

File > Open...

Image > Stacks > Animation Options...

Stack Window



- ☞ The other numbers below the title bar refer to the image's dimensions (width × height), bit depth (number of possible colors), and size (amount of computer memory the stack occupies). The size of each open image is also listed next to the image name in the bottom section of the **Window** menu.

3. How large (in kilobytes, kb) is **Galloping Horse.tif**?

Unstacking and restacking








The slices of a stack can be separated into individual windows using the **Image > Stacks > Convert Stack to Images** command. The images can be reassembled into a stack using the **Image > Stacks > Convert Images to Stack** command.

- ☞ Practice unstacking and restacking **Galloping Horse.tif**.

- Look at the bottom of the **Window** menu when **Galloping Horse.tif** is unstacked. How is it different from when the images are in a stack?
- How do the sizes of the individual images compare to the size of the stack?

- ☞ If necessary, reassemble the galloping horse images into a stack.

Editing a stack

- ☞ To draw on an image, select a line or region by using one of the selection tools (, , , , , , or ). Hold down the **F** key (for "Fill") while you drag the selection around on the image to use the selection as a paintbrush. Use this drawing technique to make changes to the images in the stack.

- ☞ Try inserting and deleting individual slices in the stack using **Image > Stacks > Add Slice** and **Delete Slice**. Experiment with holding down the **Alt** (Win) or **Control** (Mac) key while inserting slices and notice where the slice is added.

- When you add a slice with the **Alt** or **Control** key pressed, is the slice added before or after the current slice?

- ☞ Close **Galloping Horse.tif**.

Make your own movie

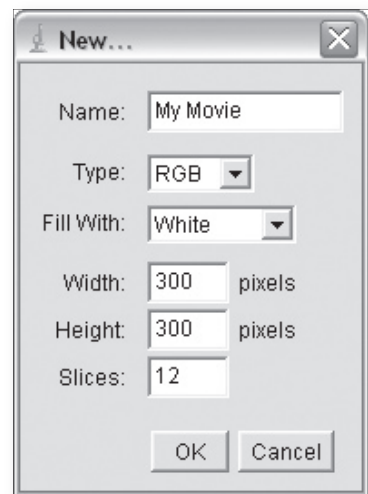
Try creating your own animation.


- ☞ Choose **File > New** to create a new blank stack named **My Movie**. To make a convincing animation, you will probably need at least 12 slices.
- ☞ On the first slice, draw a simple scene. Be sure to include an object that you want to move in the animation.
- ☞ Use the **>** key to advance to the next slice, and draw the next frame of your animation. Use the scroll bar at the bottom of the stack window to move through the stack and test your animation. (See **Drawing hints** on the next page.)








Can't see the image size?


If you can't see the size of the image in the window, try stretching the window out a bit.

Creating a new blank stack







 Here are the basic techniques you will use:

To do this...	...follow these procedures
Make a selection	Use  ,  ,  ,  , or  to select a region of interest.
Copy a selection	Choose Edit > Copy .
Paste a selection	Choose Edit > Paste .
Draw or paint on the image	Make a selection using any of the selection tools. Press the D key to draw an outline, F to fill the shape, or hold down F while dragging the selection to use the selection shape as a “paintbrush.”
Add text	Use  to enter text, then choose Edit > Draw .
Change the text or drawing color	Double-click  , and choose a new color from the palette.




 When you are finished with your animation, press **\ (Control** for Windows and **Command ** for older Macs) to start the animation. If your movie runs too slow or too fast, choose **Image > Stacks > Animation Options** to change the speed. (Movies and television both animate at a rate of around 30 frames per second.)

A stack is great for looking at a series of images one after the other. However, if you want to include the stacked images in a printed report, you need a different way to display them. A *montage* allows you to see all of a stack’s slices in a single image.

-  Choose **Image > Stacks > Make Montage** to create a montage of your stacked animation. Experiment with the settings in the montage dialog box until you are pleased with the results.
-  If possible, print your montage.
-  Save the montage and/or the stack if you like. Choose the **AVI** format to save your stack as a movie file that will play on most computers.
-  Close all open images.

“Seeing” with animation

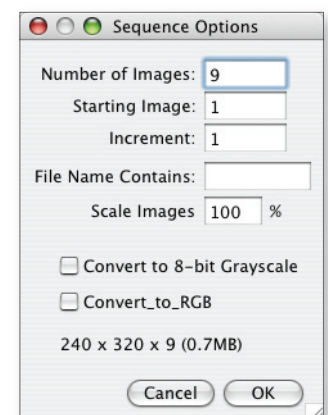
In addition to its use in the film industry, digital animation is a valuable technique widely used in other fields. Processes that occur too quickly or too slowly for the eye to perceive can be “seen” using animation. Images captured over an extended period of time can be sped up to show changes taking place that might not otherwise be noticeable. For example, meteorologists use animation to follow the movement of clouds, hurricanes, and weather fronts.

-  Choose **File > Import > Image Sequence**. Navigate to the **Hurricane Andrew** folder, and open it. In the **Open Image Sequence** dialog box, select the first file, named **About Andrew WV.tif**, and click **Open**.
 -  In the **Sequence Options** dialog box, accept the default settings, and click **OK**.
7. Describe what happens.
-  Close all open image windows and the **Log** window.

Drawing hints

You can either draw each frame of your animation from scratch, or modify a copy of the previous slice. To duplicate a slice:

- Choose **Edit > Selection > Select All**.
- Choose **Edit > Copy**.
- Use the slider at the bottom of the stack window to advance to the next slice.
- Choose **Edit > Paste**.



You have just learned an important rule for stacking a set of images—all of the images must be the same size (width and height) in order to stack them.

- ☞ Choose **File > Import > Image Sequence...** again, and open **Hurricane Andrew**. This time, in the **Sequence Options** dialog box, enter **2** for the starting image. ImageJ will now skip over the first image (the one that's a different size than the others) and should create a stack from the remaining images in the folder.

In 1992, Hurricane Andrew swept through the Caribbean, Florida, and Louisiana in 72 hours. Using animation to track the movement of hurricanes like Andrew can help provide early warnings for people seeking safety during dangerous storms.

- ☞ Animate the hurricane stack.
8. What measurements would you need to make to be able to calculate the speed of the hurricane?
- ☞ Close the hurricane stack.

In a similar manner, animation can be used to slow down events that happen too fast for us to see. Sports trainers often use animation to analyze motion such as the swing of a golfer or tennis player. Physicists study the paths of moving objects and the forces that cause them to move using animation. Biomechanical engineers sometimes use animation to help them determine the points of greatest wear when designing running shoes.

- ☞ Import the sequence of images in the **Lightning** folder.
- ☞ Animate the images.

Carefully observe these images of lightning taken during a typical Arizona summer thunderstorm. This event lasted less than two seconds.

- ☞ Use the animation slider or set the animation speed to move slowly through the stack and observe the behavior of the lightning.
9. Write down another phenomenon that occurs too quickly to “see” with our eyes. Describe how imaging could be used to slow down the event so that it can be analyzed.
- ☞ Close the **Lightning** stack.

Animation makes it easier to observe, interpret, and predict patterns.

Using stacks to show three dimensions

In the field of medicine, animation is often combined with other imaging techniques to provide noninvasive methods for visualizing anatomy.

- ☞ Import the sequence of **Brain** images in the **Makes My Head Spin** folder.

Magnetic Resonance Imaging (MRI) is a powerful way to examine the structure of a human body without cutting it open. This tool is especially useful when examining delicate structures like the human brain. An MRI is made by placing a patient in a strong magnetic field, exposing him or her to radio waves, and then recording and analyzing the way the patient's atoms respond. Unlike x-rays, which reveal bone and other hard tissue, MRI excels at creating images of soft tissue. For example, a brain tumor that cannot be detected with an x-ray might be easily identified in an MRI.

- ☞ **File > Import > Image Sequence...**

Wrong image sequence?

When importing an image sequence, the images are added to the stack in alphabetical (or numerical) order.

If the images are not named in the order that they are to be animated, you will have to open them one at a time in the correct sequence, then choose **Image > Stacks > Convert Images to Stack > Create the Animation Stack** to create the animation stack.

Remember to close any windows that are not part of the animation before building the stack.

- ☞ **File > Import > Image Sequence...**

- ☞ **File > Import > Image Sequence...**

Another advantage of MRI technology is that its images represent very thin slices through the subject, making the exact size and location of features easy to determine. The 27 images in the **Makes My Head Spin** stack show cross-sections of a human head (with no tumors, fortunately!). Images were made every 5 mm, starting from the middle of the nose and continuing up through the eyes.





Animating the brain

 Animate the **Brain** stack.

When you animate the stack, you take a trip through the brain from the nose to the top of the head. Although each individual slice has only two dimensions, the stack of slices gives us a three dimensional view.

Reslicing the brain








ImageJ can use data from the individual slices in a stack to create new views of an object. Imagine cutting straight down through the brain stack and looking at the cut from the side. This is called *reslicing*.

-  Use  to make a straight line selection across the brain image in any direction.
-  Choose **Image > Stacks > Reslice**. Set the **Input Z Spacing** to **5**, and click **OK**. (For comparison, try setting the spacing to **3**.)
-  Reslice the stack along other straight line selections drawn at various angles, and experiment with the options in the **Reslice** dialog box.

10. What structures can you see within the brain? Use the diagram at right or a textbook to help you identify and describe a few of them.

Projecting the brain

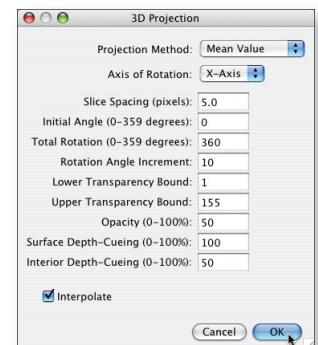
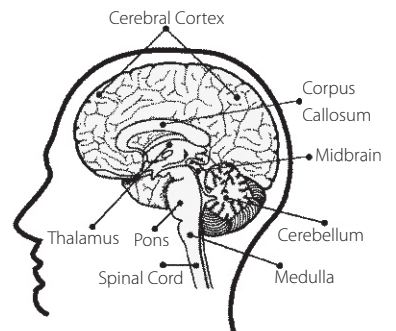
Imagine that each slice of the brain stack was printed on a sheet of clear plastic and you held the stack of plastic sheets in your hand. By tilting and rotating the stack of images, you could view them from different angles. ImageJ can create a series of images that show how a stack would appear as it is rotated around any of three axes.

-  Close all of the resliced images, but leave the brain stack open.
-  Choose **Edit > Selection > Select None** to make sure there are no selections on the brain stack.
-  Choose **Image > Stacks > 3D Project** to project the stack. Fill in the **3D Projection** dialog box as shown at right. Don't worry for now what all of these settings mean. They were chosen to allow you to look "through" the hard bone tissue of the skull at the softer, fainter brain tissue. Click **OK**.
-  To increase the contrast of the images, choose **Image > Adjust > Brightness/Contrast**, and click the **Auto** button.
-  Click the **Apply** button, then click **Yes** when asked if you want to adjust the contrast of all of the slices in the stack.
-  Close the **B&C** window.
-  Animate the completed projection stack.

Upside-down?

Your resliced brain image will be upside-down with this procedure. To make it right-side up, check the **Flip Vertically** box.

Major structures of the brain












On your own

Use the images in the **On Your Own** folder to try out the following techniques and to explore stacks and animation techniques.

Making a stack from a montage

Earlier in this lesson you turned a stack into a single image called a *montage*. You can also go the other direction, turning a montage into a stack.

-  Close all open images, and open **1 Running Horse.tif**.
-  Click , and select the first frame of the running horse.
-  Choose **Image > Duplicate** to duplicate the selection into its own window. Click **OK**. Drag the new window out of the way.
-  Choose **1 Running Horse.tif** from the **Windows** menu to activate the source image again.
-  Drag the selection outline you made to the second running horse frame.
-  Duplicate this selection. Repeat this process until you have made a separate image from each of the nine running horse frames.
-  Close the **Running Horse** montage image.
-  Stack and animate the running horse frames. Close the stack.

Registering slices in a stack

The **Rocket Stack** shows a model rocket being launched. Unfortunately, the camera moved, making it difficult to analyze the motion of the rocket. Philippe Thevenaz, a researcher of the Biomedical Imaging Group of the Swiss Federal Institute of Technology, created an amazing ImageJ add-on that automates the process of registering the images in a stack.





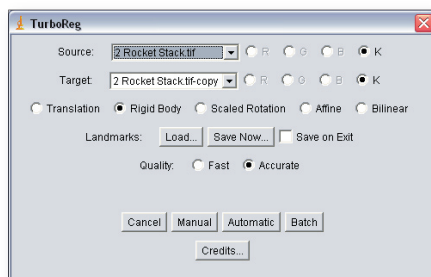
-  Open the **2 Rocket Stack.tif** image.
-  Choose **Image > Duplicate** to make a copy of the first slice of the stack (not the whole stack, though it will still work if you do.)
-  Choose **Plugins > TurboReg > TurboReg**.
-  Use the default settings, and select the **Accurate** option.


Image registration—correcting for differences in position of features in two or more images due to camera motion and other effects.

Note to Mac OS 9 users—TurboReg is only available for Windows and Mac OSX.



-  Click the **Batch** button.

The plugin takes over and registers the stack!

-  Animate the registered rocket stack. To compare the stacks before and after the registration process, you can click on one of the original rocket stack copies and animate it.



Lights, Camera, Action!

1. What are the fastest and slowest animation speeds in frames per second?
 - a. fastest = _____ fps (frames per second)
 - b. slowest = _____ fps (frames per second)
2. How many slices are there in **Galloping Horse.tif**?
3. How large (in kilobytes, kb) is **Galloping Horse.tif**?
4. Look at the bottom of the **Window** menu when **Galloping Horse** is unstacked. How is it different from when the images are in a stack?
5. How do the sizes of the individual images compare to the size of the stack?
6. When you add a slice with the **Alt** or **Control** key pressed, is the slice added or before or after the current slice?
7. Describe what happens.
8. What measurements would you need to make to be able to calculate the speed of the hurricane?
9. What do you notice about the path the electricity travels?
10. What structures can you see within the brain? Use a textbook to help you identify and describe a few of them.