**Imaris 4.2 user information**

There is also a manual to help you use Imaris. It is on the shelf above the Windows machine, to the left of the Adobe box. Please make sure you return it there when you are finished.

- To zoom in and out click down on the rollerball and drag the mouse
  To pan the image press down the right button and drag the mouse.
  You can also flip and rotate the x, y, or z axis of your object from the Image Processing menu.

- To rotate / spin the object in **Surpass mode**. Click and drag with the left mouse button.
  To zoom in and out of the object in **Surpass mode**. Click and drag with the left mouse button and your rollerball clicked down.

- If you need to you can change the color of channels:
  Goto Edit > Image Properties > Channels, select the channel which color you want to change
  Click in the Base Color field to select a new color
  Click OK

- To open a file:
  File > Open > All files
  It is easier to choose the **all files** option if you are going to be opening files from a variety of microscopes. Imaris will be able to open any file types. If you select a specific microscope type, Imaris will remember that setting next time you want to open an image.

- To make sure you get the right colors:
  Goto Edit Menu  > Preferences > Loading
  Choose Take Colors from > File colors or Emission Wavelength

- There are a few working modes that you will use in Imaris. The default mode when you open an image is **Slice**. However, if you are working in another mode, for example, Surpass, when you open another image all the calculations currently displayed in Surpass will be applied to the newly opened image. This can lead to loss of time when you re-edit, and will also take up more memory. Remember each time you open a new image to return to Slice mode.

- When working with your image you can choose a rendering mode. This is either MIP or Blend. MIP shows the brightest pixel in the Z direction, this produces a very bright image - the background is turned to black. Blend makes your stack opaque and allows shadows for a 3D environment.
  To see shadows you will need to set the background to a bright value:
  Edit Menu > Preferences > Display Menu > & set the Background color. In the rendering box for blend select Light & Left or Right.
  Often in Blend mode the object can be surrounded by a dark halo, this is the background noise illustrating low intensity signals. You can remove this by increasing the threshold value in the **Volume Properties** panel.

To access online training for Imaris:
www.bitplane.com > goto the menu bar on the left side > Training
Scroll down to > Visit Our WebEx Archive & Click on Training Archives
You will be asked for a username and password:
User: imug
Password: im405

All the archived tutorials are listed > choose the one you would like to watch. You will need to download software. I took screenshots from the > **Imaris Getting Started Overview**. Click to select > you will probably need to type the username and password again.
**Adjust Brightness / Contrast**

Go to the **Edit menu** to open the **Display adjustment** window. If there is just one channel you will only see 1 channel. The example below shows 3 channels. Click the box on the left side to activate that layer.

There are also two sliders on the top right and left of each channel, which will lighten / darken your object.

These lower advanced settings will also give you control over how your object is adjusted. With this slider you can adjust the transparency.

**Smoothing Filters**

To best learn how these filters work practice using them. To use the smoothing tools:

Goto Image Processing > Image Smoothing > Gaussian, Median, Edge Preserving

- **Gaussian.** One voxel’s intensity is replaced by the weighted sum of the intensities of its neighbours. The weights in any direction correspond to a bell curve centered at the voxel’s position.
- **Median.** One voxel’s intensity is replaced by the median intensity of its neighbours.
- **Edge Preserving.** Similar to Gaussian but instead of filtering all directions it only filters in directions without gradient.

**Images where smoothing filters have been applied.**

- **Top:** Original single section.
- **Middle:** Image filtered with Gaussian filter.
- **Bottom:** Image filtered with edge preserving filter (EPF) using same filter width than for Gaussian filter.
- The EPF is not a standard kernel operation. Instead the filter kernel is computed newly for each voxel position to avoid filtering across edges.
- **Benefit:** While the noise removal effect (smoothing) of the EPF is similar in areas of more homogenous intensities it does not filter across intensity gradients (edges).
- **This filter preserves the overall intensity in the image.** The integral of all voxel intensities remains constant.
Another useful tool is Background Subtraction. This is similar to the Unsharp Mask filter in Photoshop. Background Subtraction can sharpen an image - as shown in the bottom image. This filter enables you to define objects more specifically.

To access Background Subtraction:
Image Processing > Image Thresholding > Background Subtraction

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1. **Background Subtraction**

   - The default filter width is set so that only very broad intensity variations are removed from the image. This effect can be used to remove bleaching or shading (see bottom image pair).

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2. **Background Subtraction**

   - If smaller filter widths are chosen then the filter has edge detection and line detection properties. The figure shows microtubules (line objects) and kinetochores (point objects) before (top) and after filtering (bottom).
   - The filter width for the microtubule bundles (green) has been chosen to 0.2 μ, the filter width for the kinetochores to 0.3 μ.
The Surpass view mode is probably where you will spend much of your editing time. The Surpass view allows interaction with the objects. To activate the Surpass View Click on the Surpass button on the top of your image.

**Surpass View: Unique 3D Work Place**

- The Surpass view that Biplane invented in 2001 is the heart of Imaris.
- It allows users to identify objects and then work with them in a natural way: namely by first selecting and then inspecting or modifying the objects.
- Each object has its own creators, editors, and inspectors, and provides its own statistics (the latter with MeasurementPro only).
- Surpass allows dual interaction with the scene and the list and enables mixed-model rendering where one object can be a surface and another can be shown as voxels.
- Using the Imaris scene file you can store your data with all the objects that you created. This is a good way to keep your work close to the original data.

If you just have one channel in your image you can use the navigator controls, but you cannot select any objects. Instead you can create 3D objects to replace the cells in your image, and then you can control the color and have more editing control. These objects are called Spots, they are discussed on the next page.
If you want to create a more 3D view of your object you can create spots if you just have one channel.

Click the **Add New Spots** button.

If you do not see this button:

Goto the **Surpass menu bar > Add New > Spots**

You will now see the Spots Properties window shown above the Objects window.

You need to know the measurement of each of the cells, or the average size. If you don’t know the size of the cells, you can measure them.

Click on the **Slice button** at the top of the screen.

Click on the measurements button on the right.

Choose a cell, click and drag across the cell as shown in the image to the left. The measurement will be shown at the bottom of the screen.

You can also measure multiple cells. In Imaris 4.2 you can specify between two different measurements, and spots will be applied to all cells between those two measurements. This is called **Multiscale Spots.**
If you want to turn off the original cells to see how the “Spots,” look just click the checkbox next to the volume layer.

Once you have set the measurement(s), click the Next button at the bottom of the Properties window. This will take you to the Threshold properties. On the screen you will see that spheres now represent the cells with the measurements you specified.

If you move the Threshold slider to the right smaller cell measurements will be revealed, if you move it to the left, only the larger cells will be shown.

If you want turn off the original cells to see how the “Spots,” look just click the checkbox next to the volume layer.

Once you are happy with the “Spots,” Click the Finish button.

In the Surpass mode you can also create ISO surfaces, these can be “smooth,” or “accurate.”
Using Surpass mode to create movies.

You will need to learn the basics of animation to put your slides into a movie presentation, but Imaris makes it easy for you. In the Surpass mode you can create Keyframe animations which can be saved as .AVI, or .MPEG. All these formats can be used in Powerpoint presentations or the .MPEG can be edited/viewed in Quicktime, if you save it from Quicktime as a .MOV you can then open it in iMovie.

Keyframe animation
Understanding Keyframes is fundamental to understanding how animations are put together. The article below explains the terminology and the basics of animation.

In Imaris you set-up the Keyframes, and Imaris calculates the in-between frames.

To set-up the keyframes you need to be in the Surpass Mode. Check the Animation box found to the right of the surpass button.

Using the Keyframe animation panel you will need to set-up the number of frames in your movie. Normal video speed is 25 frames per second (FPS). If your animation has 4-5 keyframes you will need around 400 frames, which should last for around 16 seconds. Click on settings to choose the frame rate and also to decide if your movie will play once or loop continuously.
To create the keyframes, move, scale, or rotate the scene to where you want and click Add in the Keyframe panel. Move your scene to the next keyframe view and click Add again. Continue until you have finished. In the time sequence panel of the Animation box there will be blue bars. These represent the key frames, they should be distributed evenly through this panel which will make a smooth movie. If they do not look even click the Auto Distribute button.

To preview your movie click the play button. Make any changes that are necessary, when the movie is finished, it must be recorded - click the record button.

Before the movie is recorded you need to decide how you want to save your movie. If you will be working on a Mac save the movie as an MPEG file type, if you are working on a Windows platform, save the movie as an AVI file type.

You also need to decide whether you want a high quality movie with low compression, or a high compressed movie with low quality. The lower the quality the smaller the file size. Movie clips can be memory intensive. For example an 11 second movie with the compression settings shown in the image to the left was 11MB.

Please note: It can take a while for the movie to record, it will record much slower than it plays.