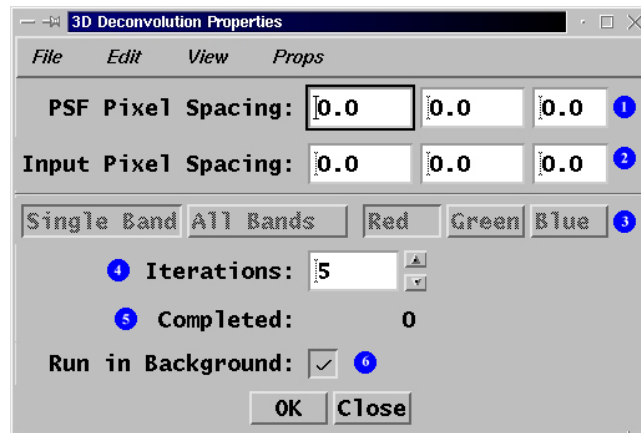


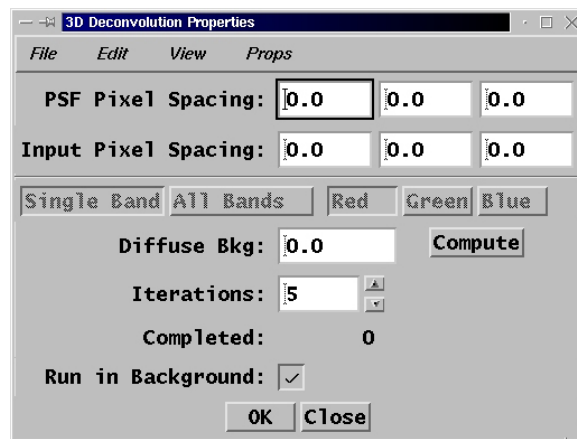


ESee 3D Deconvolution Properties Screen



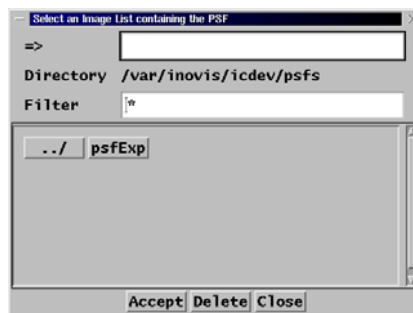
- ① **PSF Pixel Spacing** - displays the X, Y, and Z pixels spacing of the PSF images.
- ② **Input Pixel Spacing** - displays the X, Y, and Z pixel spacing of the input images.
- ③ **Band: Red, Green, Blue** - enabled when RGB images are the input source
- ④ **Iterations** -allows the user to set the number of iterations (passes) that the deconvolution algorithm will make on each input stack.
- ⑤ **Completed** - displays the number of iterations that have been completed.
- ⑥ **Run in Background** - when , the deconvolution process is run in the background allows the user to perform other imaging functions.

ESee 3D Deconvolution Properties Screen (continued)

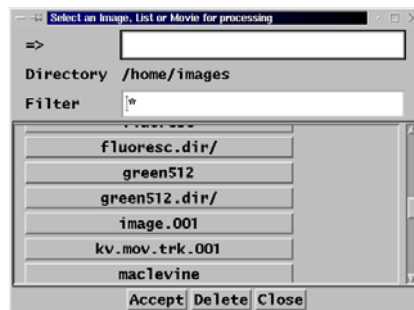


File Menu

Open PSF Images Function - opens the PSF image directory



Open Input Images Function - opens the Input image directory

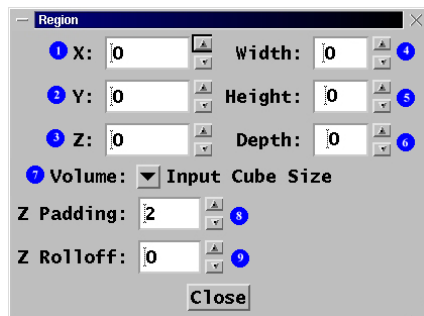


Reset Result to Input Function - copies the input images to the current output stack.

ESee 3D Deconvolution Properties Screen (continued)

Edit Menu

Region Function - allows the user to select the cube size for the resultant deconvolved images



- ① X: - displays the X pixel spacing
- ② Y: - displays the Y pixel spacing
- ③ Z: - displays the Z pixel spacing
- ④ Width: - displays the volume width
- ⑤ Height: - displays the volume height
- ⑥ Depth: - displays the number of images in the volume
- ⑦ Volume - allows the user to select the volume
- ⑧ Z Padding - creates extra images on the top and bottom of the stack to reduce the effect when the top and bottom images are not approximately the same.
- ⑨ Z Rolloff - attenuates the top and bottom images with a cosine function to approximate a periodic function in Z.

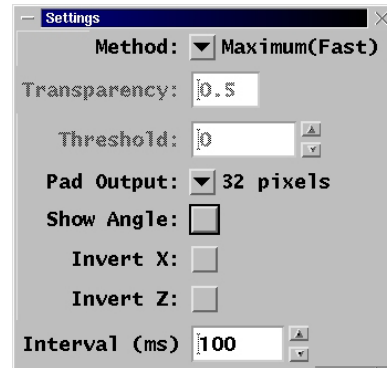
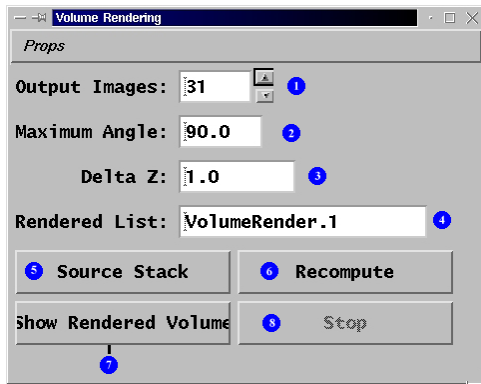
ESee 3D Deconvolution Properties Screen (continued)

View Menu

PSF Images Function - allows the user to view the current PSF image list

Input Images Function - allows the user to view the input image list

3D Render Output Function - allows the user to create a 3D rendering of the deconvolved images.



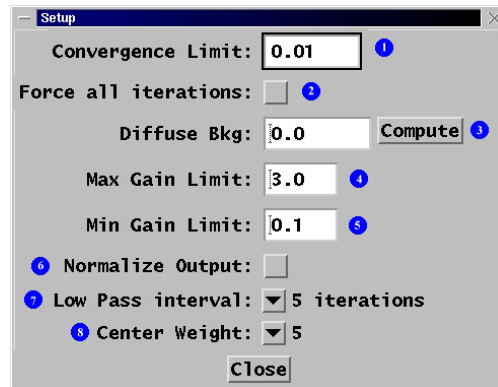
Props Subfunction-

- ① **Output Images** - allows the user to set the number of output images
- ② **Maximum Angle** - allows the user to set the maximum angle of the rotation
- ③ **Delta Z** - allows the user to set the Z spacing
- ④ **Rendered List** - allows the user to input the output list file name
- ⑤ **Source Stack** - allows the user to input the source stack file name
- ⑥ **Recompute** - computes the 3D rendering
- ⑦ **Show Rendered Volume** - displays the rendered volume
- ⑧ **Stop** - stops the 3d rendering before completion

ESee 3D Deconvolution Properties Screen (continued)

Props Menu

Setup Function



- 1 **Convergence Limit** - Each pass of the deconvolution computes the residual (RMS[observed - computed]) error. If this residual error does not change, then the result has converged to a limit. This property determines how much the residual needs to change to continue the next iteration.
- 2 **Force all iterations** - Ignores the convergence test and completes all requested iterations.
- 3 **Diffuse Background** - scans the input images, finds 100 pixels with the lowest gray levels, and computes their mean. This function is intended to prevent reassignment of diffuse fluorescence as out-of-focus photons. If a fluorescent structure is immersed in a fluorescent solution, a dark "halo" will be generated around the structure by incorrectly assigning photons from the solution to the structure.
- 4 **Max Gain Limit** - With each iteration, an estimate of the deconvolved data is convolved with the PSF and compared to the observed data. The ratio of the observed / convolved (estimate) is used to make a guess for the new estimate. This property in conjunction with 4 sets the upper limit of this ratio to prevent noisy pixels from increasing or decreasing too rapidly.
- 5 **Min Gain Limit** - With each iteration, an estimate of the deconvolved data is convolved with the PSF and compared to the observed data. The ratio of the observed / convolved (estimate) is used to make a guess for the new estimate. This property in conjunction with 5 sets the lower limit of this ratio to prevent noisy pixels from increasing or decreasing too rapidly.
- 6 **Normalize Output** - When the resulting output data exceeds the range of the storage, ie, gray levels greater than 65535, enables re-scaling to save these values within the output precision.

ESee 3D Deconvolution Properties Screen (continued)

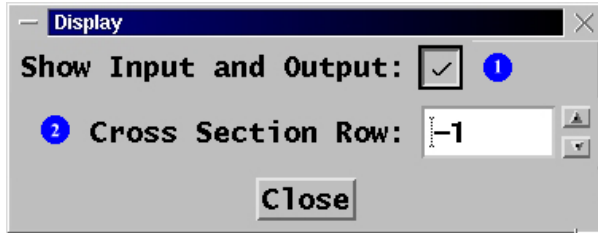
Props Menu

Setup Function

- when iteration
- ⑦ **Low Pass Interval** - Since the noise in the resulting data gets treated as high-frequency (sharply focused) objects, it frequently helps to perform a low-pass (smoothing) operation on the resulting data. The "Low Pass Interval" determines this low-pass operation is performed; each iteration, every other (2), every third iteration (3), and so on.
 - ⑧ **Center Weight** - the "Center Weight" is the ratio of the center pixel to its neighbors. A weight of 1 treats the center equally with its neighbors and gives the most smoothing (blurring). Higher weights produce less blurring.

ESee 3D Deconvolution Properties Screen (continued)

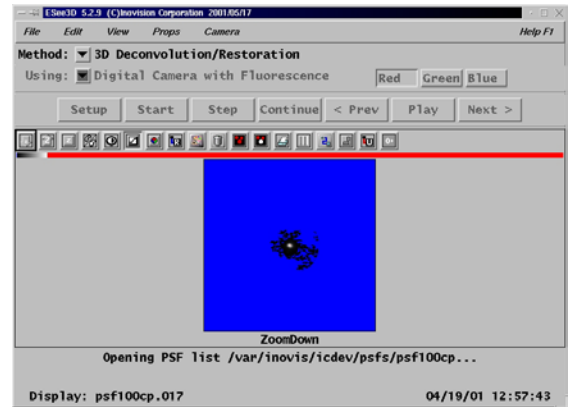
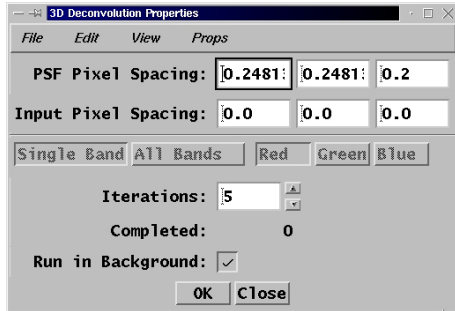
Display Function



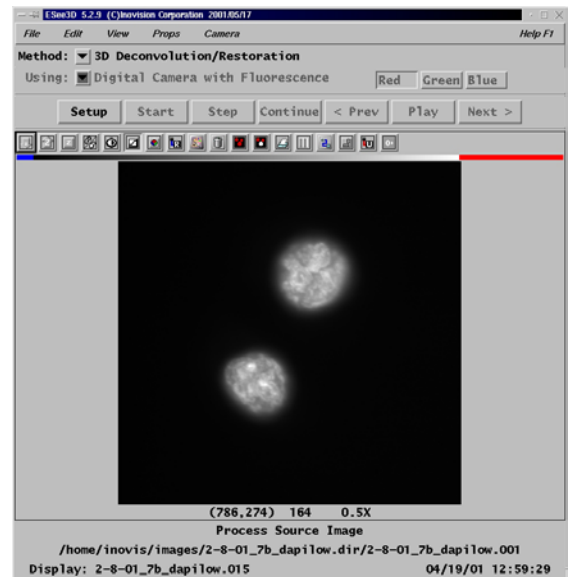
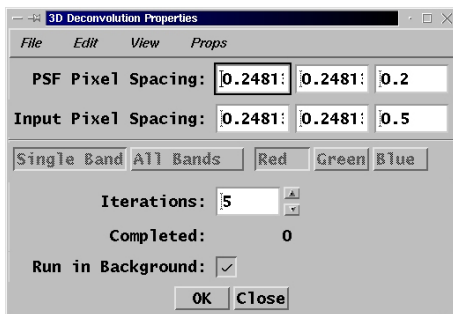
- ① **Show Input and Output** -when \surd the input and output images are displayed side by side in the ESee 3D Image Window.
- ② **Cross Section Row** - allows the user to select the cross section row to be displayed as the images are being deconvolved.

ESee 3D Deconvolution Getting Started

1. From the File Menu, Select "Open PSF Images" Function.
2. Select the appropriate PSF List and click on "Accept".

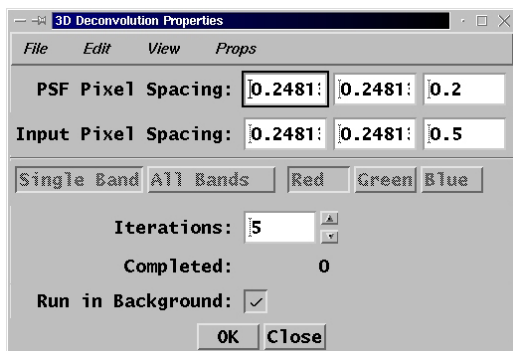


3. From the File Menu. Select "Open Input Images" Function.
4. Select the appropriate Input Image List and click on "Accept"

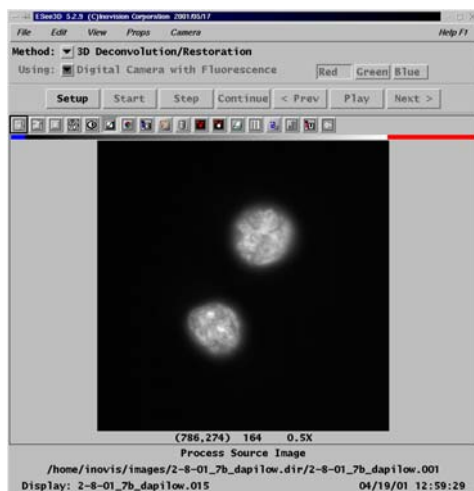


ESee 3D Deconvolution Getting Started

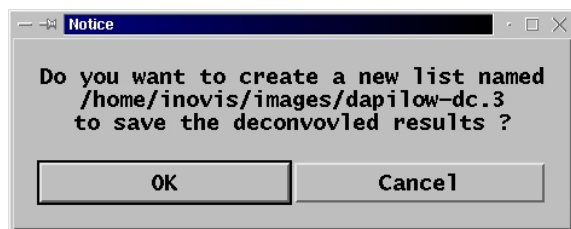
5. From the "Iterations" Function on the main screen, set the number of iteration to be deconvolved.¹
6. Click on the "OK" button



7. From the ESee Main Screen, click on the "Start" button.



8. You will then be prompted to create a new image list to save the deconvolved images. If you click on the "OK" button, a new image list will be created with a -dc extension.



ESee 3D Deconvolution Getting Started

9. The deconvolution process will then start. The original image will appear on the left hand side of the screen and the deconvolving image will appear on the right hand side of the screen. At the bottom of the image will be the progress status.

