Introduction

SimplePCI **D**econvolution **DNN**) has two options **D**econvolution **D**eblur (**DNN-D**) and **D**econvolution **2D** Blind (**DNN-2D**). **DNN-2D** uses restorative deconvolution algorithms to identify out-of-focus haze and restore it to its original point. A **P**oint-**S**pread **F**unction (**PSF**) is automatically derived from an image and used in restoration. **DNN-D** deblurs images based on algorithms of no neighbor and nearest neighbor to remove haze from optical image sections. Out-of-focus haze is mathematically modeled from an estimated **PSF** to deblur an image. **DNN-D** supports nearest neighbor and no neighbor algorithms.

DNN supports the following features:

- Remove or Restore blur in images using the fast algorithms
- Apply to individual, multi-wavelength or image sequences
- Deconvolve multi-wavelength images
- Easy access to deconvolution settings
- Automatic calculation of point-spread functions (**PSF**)

Expand the functionality of ***SimplePCI*** and **DNN** with optional modules:

- **AIC**, automated control and image acquisition
- **DIA**, dynamically measure intensity changes on or off-line
- **IPA**, develops icon-driven work files for automatic image analysis and processing
- **IPA-MTA**, track and analyze moving objects
- **QFA-FRET**, accurate FRET measurements and cross talk correction
- **VIS-MD**, provides rapid 3D visualization of multi-dimensional data sets

Getting Started

This **Quick Start Guide** contains examples of how to utilize **DNN**. For further assistance, refer to the online help, manual, or visit support at http://www.cimaging.net, for access to the latest **How to's** and frequently asked questions. Additional support is available at e-mail: support@cimaging.net, or Tel: 412-741-7920.

Example guides:

Restore an Image or Data Document using 2D Blind Deconvolution

Module Required DNN-2D

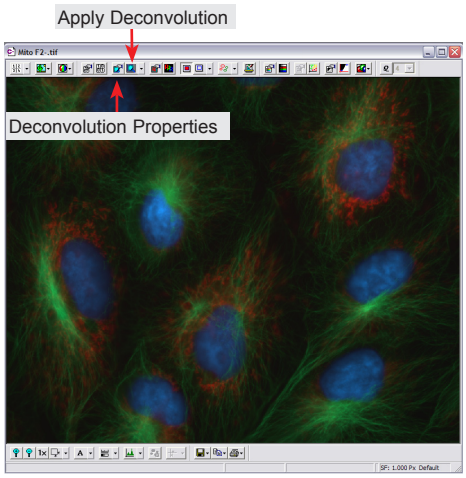

fig.1

1. Open an image or data document.
2. Click the down-arrow next to the **Apply Deconvolution** icon and select **2D Blind** (**fig.1**).
3. Click the **Deconvolution Properties** icon.

   Note: CPU usage is high during deconvolution; therefore, we recommend exiting other applications. ***Hint!*** crop a small area in an image to test appropriate settings, then apply to full image.
4. Enter number of iterations for each color in the R,G,B Iterations section. The number of iterations varies from sample to sample, adjust until a satisfactory result is found. Start with a high value of 20 and note which number corresponds to the best image; use this number for similar images. To speed up process, select a PSF from a channel and apply to other channels. Alternatively, select a PSF from a field and reuse for other fields. **Background Correction** should be applied for best results, provided there is real background in the image.
5. Click **Apply** button to review the result. Modify the number of iterations as required. (**fig.2**).
6. Save settings for future use by clicking on **Add** and assigning a name (**fig.2**).
7. Click on **OK** to start the deconvolution process.
8. Click the **Apply Deconvolution** icon to toggle between processed and non-processed image, and compare the effect of deconvolution (**fig.1 & 3**). If contrast has been applied, it may cause an image to appear bright . Undo contrast to view image normally.

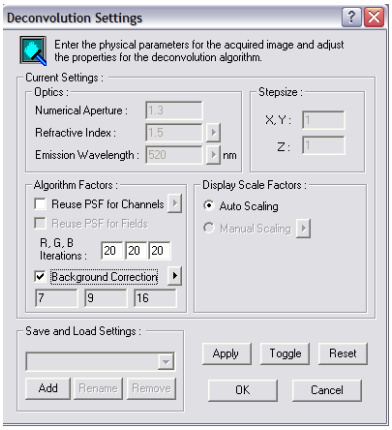

fig.2

DNN-2D operates the same on data documents containing a sequence of images. To export restored images to another file, right click on an image, select Export Image Sequence –Processed Image. Choose from a new data document, AVI, Tiff images, or Multi-page Tiff for the new format. Clicking on Play button offers Export Image sequence as an option.

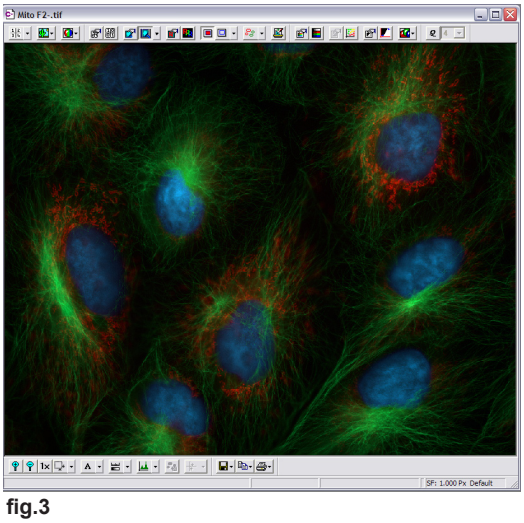
fig.3

Deblur an Image using No Neighbor

Module Required DNN-D

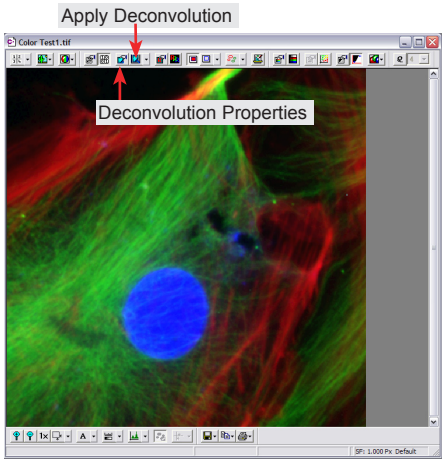

fig.4

1. Open an image.
2. Click the down-arrow next to the **Apply Deconvolution** icon and select No Neighbors (**fig.4**).
3. Click the **Deconvolution Properties** icon.
4. Enter microscope conditions for the image to be restored. Settings must be the ones used when the image was captured (**fig.5**).
   - **Numerical Aperture**, enter **NA** of the objective
   - **Refractive Index**, select media used during acquisition
   - **Emission Wavelength**, enter emission wavelength for fluorophore
   - **XY** field, enter calibration scale factor applied to each pixel. The scale factor is derived from the calibration file. If the scale factor is not known, see page 5 of the ***SimplePCI* Quick Start Guide**
   - **Z** step size, enter Z step increment between images used during Z scan of the sample. Use default values for the remaining fields, and adjust until satisfied

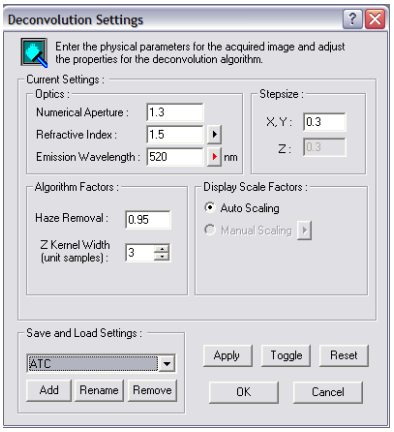

fig.5

5. Click **Apply** button to review the result. Adjust **Haze Removal** to control the degree of deblurring. **Z-Kernel Width** determines the number of slices above and below the focal plane considered in the estimation of out-of-focus (**PSF**). **Caution**: too much haze removal may cause undesirable noise with loss of true structure.
6. Save settings for future use by clicking on **Add** and assigning a name.
7. Click on **OK** to start the deconvolution process.
8. Click the **Apply Deconvolution** icon to toggle between processed and non-processed image, and compare the effect of deconvolution (**fig.4 & 6**). If contrast has been applied, it may cause an image to appear bright . Undo contrast to view image normally.

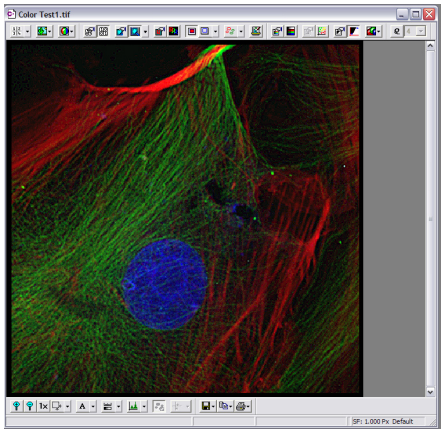
fig.6

DNN-D no neighbor operates the same on data documents containing a sequence of images. To export restored images to another file, right click on an image, select Export Image Sequence, Processed Image. Choose from a new data document, AVI, Tiff images, or Multi-page Tiff for the new format. Clicking on Play button, offers Export Image sequence as an option

Deblur Images in a Data Document using Nearest Neighbors

Module Required DNN-D

1. Open a data document and select an image from a field.

2. Click the down-arrow next to the **Apply Deconvolution** icon and select Nearest Neighbors (**fig.7**).

3. Click the **Deconvolution Properties** icon.

4. Enter microscope conditions for the image to be restored. Settings must be the ones used when the image was captured (**fig.8**).

   - **Numerical Aperture**, enter **NA** of the objective
   - **Refractive Index**, select media used during acquisition
   - **Emission Wavelength**, enter emission wavelength for fluorophore
   - **XY** field, enter calibration scale factor applied to each pixel. The scale factor is derived from the calibration file. If the scale factor is not known, see page 5 of the ***SimplePCI* Quick Start Guide**
   - **Z** step size, enter Z step increment between images used during Z scan of the sample. Use default values for the remaining fields, and adjust until satisfied

5. Click **Apply** button to review the result. Adjust **Haze Removal** to control the degree of deblurring. **Z-Kernel Width** determines the number of slices above and below the focal plane considered in the estimation of out-of-focus (**PSF**). **Caution**: too much haze removal may cause undesirable noise with loss of true structure.

6. Save settings for future use by clicking on **Add** and assigning a name.

7. Click on **OK** to start the deconvolution process.

8. Click the **Apply Deconvolution** icon to toggle between processed and non-processed image, and compare the effect of deconvolution (**fig.7 & 9**). If contrast has been applied, it may cause an image to appear bright. Undo contrast to view image normally.

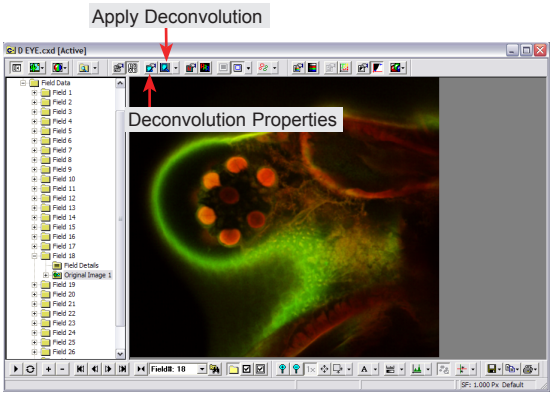

fig.7

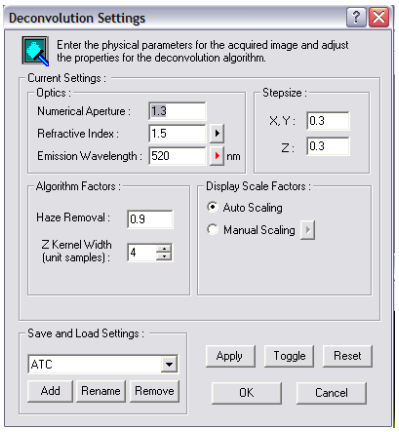

fig.8

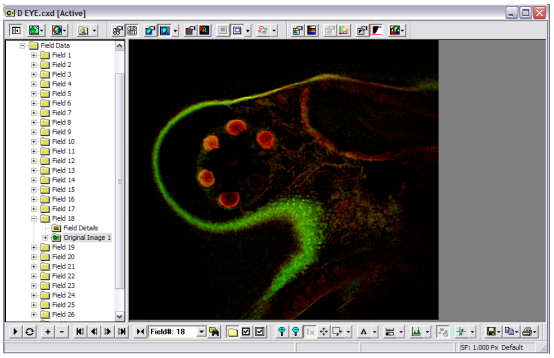
fig.9

DNN-D nearest neighbor operates on data documents containing a sequence of images. To export restored images to another file, right click on an image, select Export Image Sequence, Processed Image. Choose from a new data document, AVI, Tiff images, or Multi-page Tiff for the new format. Clicking on Play button offers Export Image sequence as an option.