

## TOF/TOF™ Imaging Acquisition Software V1.0

### Features

The TOF/TOF™ Imaging Acquisition Software allows the user to easily setup and perform data acquisition on imaging samples using the AB SCIEX TOF/TOF™ 5800 System.

The software allows the user to visualize the sample on the MALDI plate and to graphically specify spots for acquisition. You can select a fixed position or continuous motion acquisition method and specify a Data Folder and Image file name. These acquisition settings and regions of interest can be saved to a file for future use.

Data is saved to the specified data folder in the Analyze 7.5 format and can be processed in the AB SCIEX TissueView™ Software.

The TOF/TOF Imaging Acquisition Software is installed alongside the currently installed TOF/TOF Series Explorer Software. The Imaging software uses the TOF/TOF Series Explorer Software and the Remote Data Access Software subsystem to control the instrument hardware and to acquire data from the instrument.

### Where to Get Help

If you have any questions or need assistance with this procedure, contact AB SCIEX Technical Support:

- For telephone support, visit <http://www.absciex.com> and then click Contact Us.
- For application questions, visit [www.absciex.com/FAQ](http://www.absciex.com/FAQ)
- Email: [support@absciex.com](mailto:support@absciex.com)

### Requirements

AB SCIEX TOF/TOF™ 5800 System

TOF/TOF™ Series Explorer™ Software

### Supported Equipment

NA

### Installation Instructions

Two software packages must be installed on the TOF/TOF instrument acquisition computer.

1. To install the Remote Data Access software, double-click **setup.exe** in the **Remote Data Access** folder and then follow the installation instructions on the screen.

2. To install the Imaging Acquisition software, double-click **setup.exe** in the **TOFTOF Imaging Acquisition** folder and follow the instructions on the screen. The TOF/TOF™ Imaging Acquisition Software is installed by default in the C:\Program Files\AB SCIEX\TOFTOF\Imaging Acquisition folder.

## Licensing Instructions

1. When the imaging software is first started, the software activation screen is shown. The software must be licensed in order to fully create, save, and submit an imaging job.

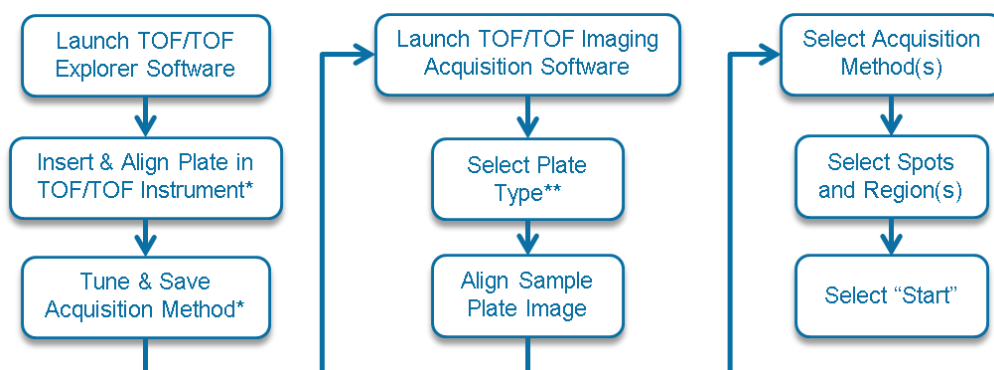
**Note:** If the software displays a status of unlicensed, you can install the license file from the Configuration screen.

2. Follow the instructions on the Software Activation screen.

3. Upon exiting the Software Activation screen, the software shows the license expiration.

**Note:** This can also be seen in the About screen.

## High Level Workflow



Steps denoted by a single asterisk (\*) can be performed at any time before selecting “Start” in the TOF/TOF Imaging Acquisition Software. Steps denoted by a double asterisk (\*\*) are optional.

## Detailed Workflow

1. Create a graphical image of the MALDI plate and sample to be used with the TOF/TOF™ Imaging Acquisition Software (.bmp, .jpg, .tiff, .gif, .png).

**Note:** To successfully navigate the Alignment Wizard, make sure the alignment marks on the image are not skewed. You may need to re-create the plate image by re-scanning it or rotate the image using an editor such as Microsoft Paint or Microsoft Picture Manager.

2. In the TOF/TOF™ Series Explorer™ Software, do the following

- Load and align the sample plate.
- Ensure Interactive mode is selected.

3. Launch the TOF/TOF Imaging Acquisition Software.

4. Click **Configuration > Plate Template** and then select the required Plate template.

**Note:** The Sample plate type cannot be changed after the Plate Image Alignment is started

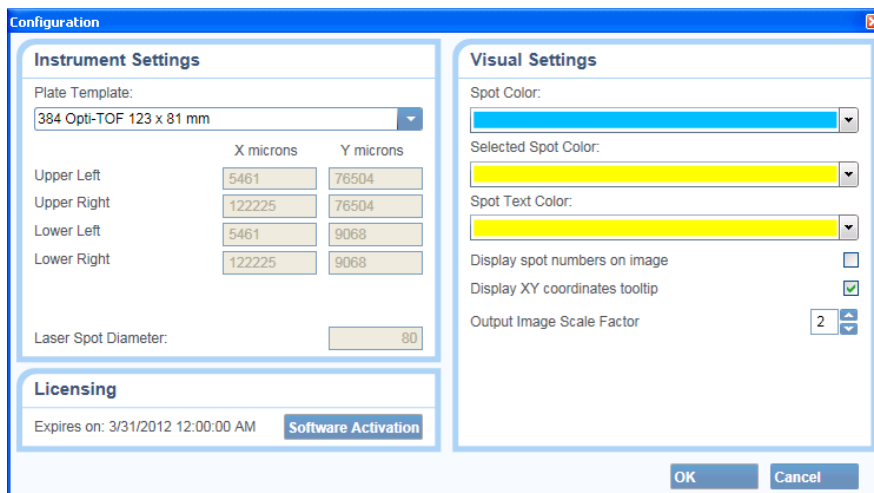


Figure 1. Configuration window with 96 Opti-TOF 123 x 81mm plate template selected.

5. Select **Import Image** and then navigate to the required plate image (.bmp, .jpg, .tiff, .gif, .png). After the file is selected, the unaligned image is shown.

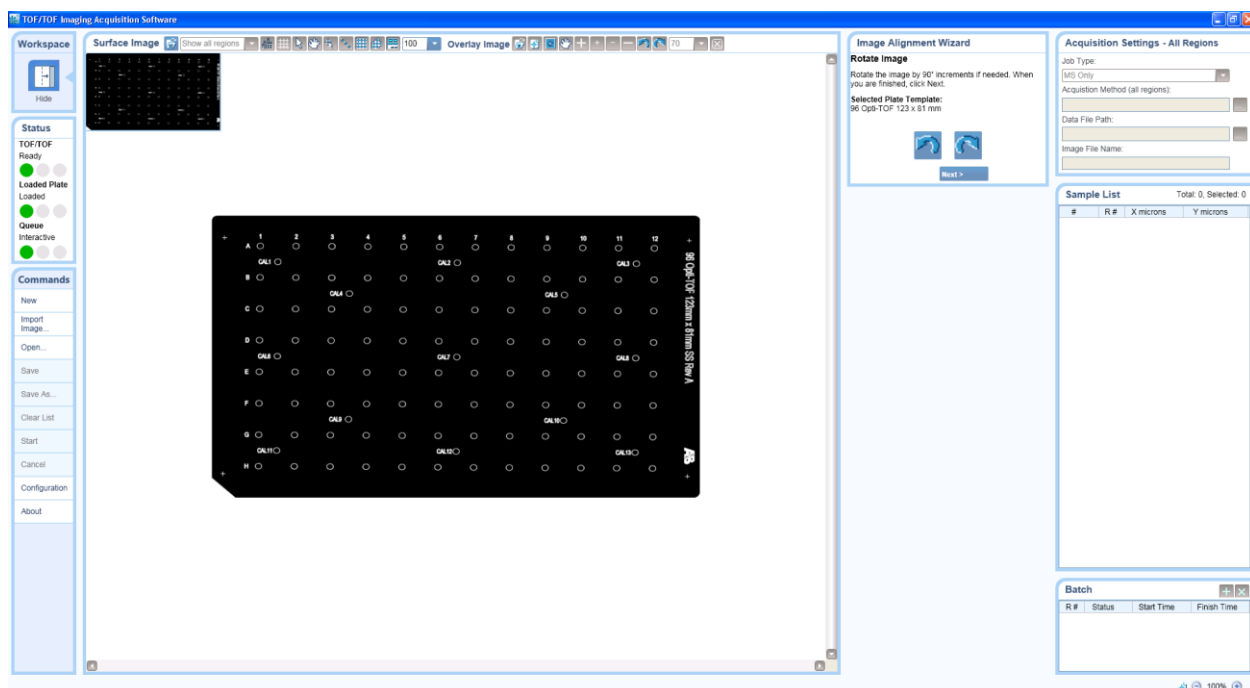


Figure 2. 96 well plate image imported and Alignment Wizard open.

6. Follow the instructions on the **Image Alignment Wizard** screens, selecting the alignment marks on the graphical plate image.
7. Click **Next**.

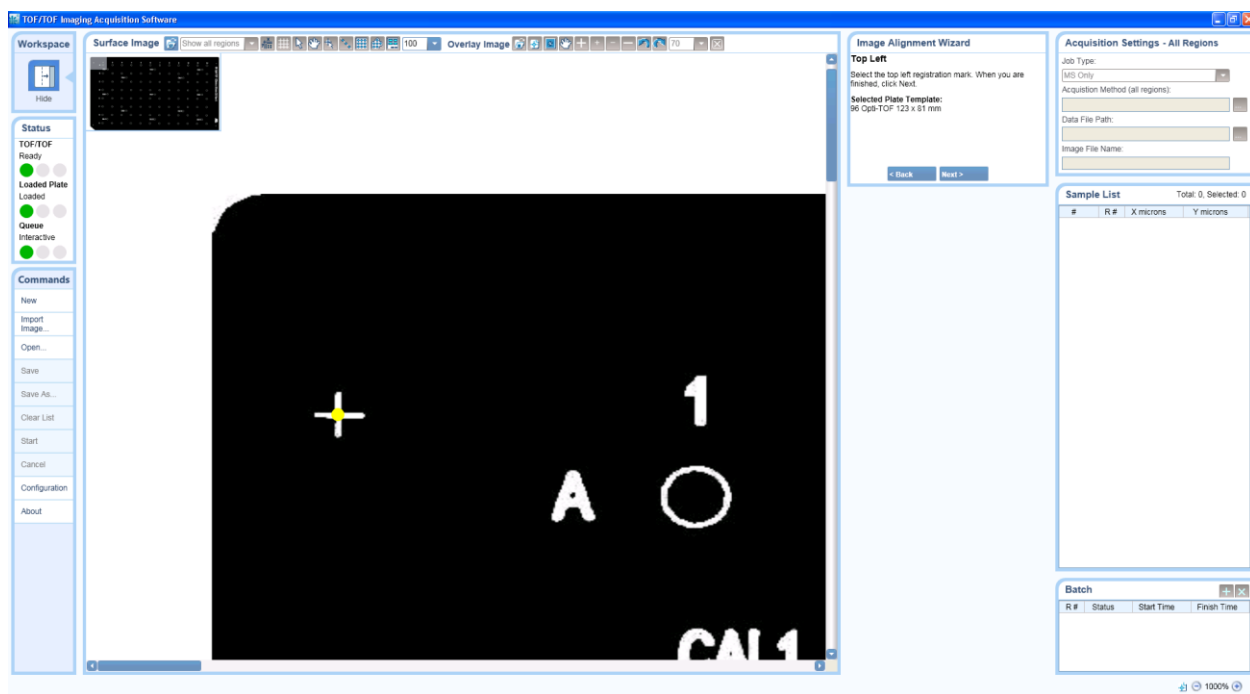


Figure 3. Alignment Wizard with Top Left alignment mark selected.

8. Upon completion of the Alignment Wizard – **Region 1** is created and is shown in the Batch list.

Note: Use the Move To Position When Available toolbar button and the Video Viewer in TOF/TOF™ Series Explorer™ Software to confirm the graphical image alignment. Click on the image in the Imaging Acquisition Software and verify the sample stage moves to the correct location. If the position is not correct, make sure that a plate alignment within the TOF/TOF Series Explorer Software was performed correctly and the plate type selected in the TOF/TOF Imaging Acquisition software is correct.

9. Select the spacing level between spots from the drop-down list in the toolbar.
10. Add spots to the image using the toolbar drawing tools for individual spots, a line of spots, rectangular or irregular areas. A rectangular area of spots can also be added by manually entering XY coordinates using a toolbar button. The Sample List is populated with the spot positions as the spots are added.
11. Enter **Acquisition Settings** by selecting an Acquisition Method, a Data File Path and typing an Image File Name.

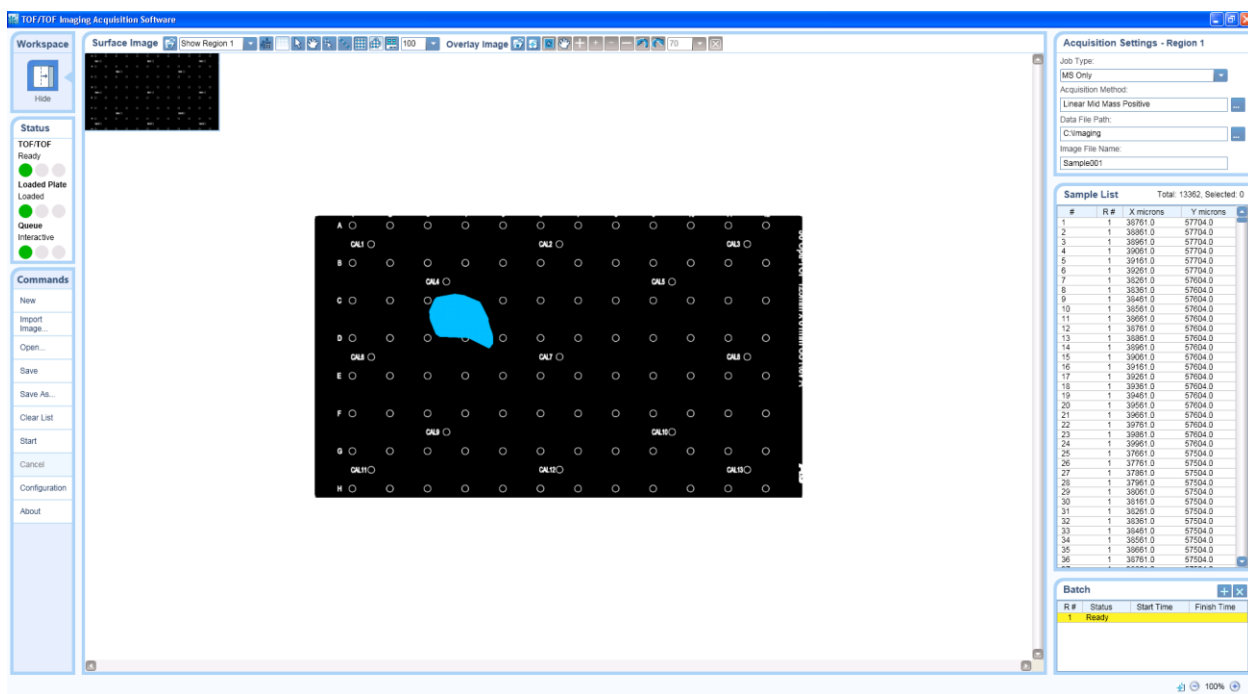


Figure 4. Image with irregular area of spots added.

12. Click **Save** or **Save As...** to save the acquisition settings (\*.IAS file) for future use.
13. Optionally - multiple regions can be created by selecting **Create New Region** in the image display menu or the batch list. The Job Type and Acquisition Method can be specified individually for each region. To remove a region, select the Delete Region button in the batch list.

**Note:** The region number suffix is appended to the data file for each region: (for example, My Data File-Region 1).

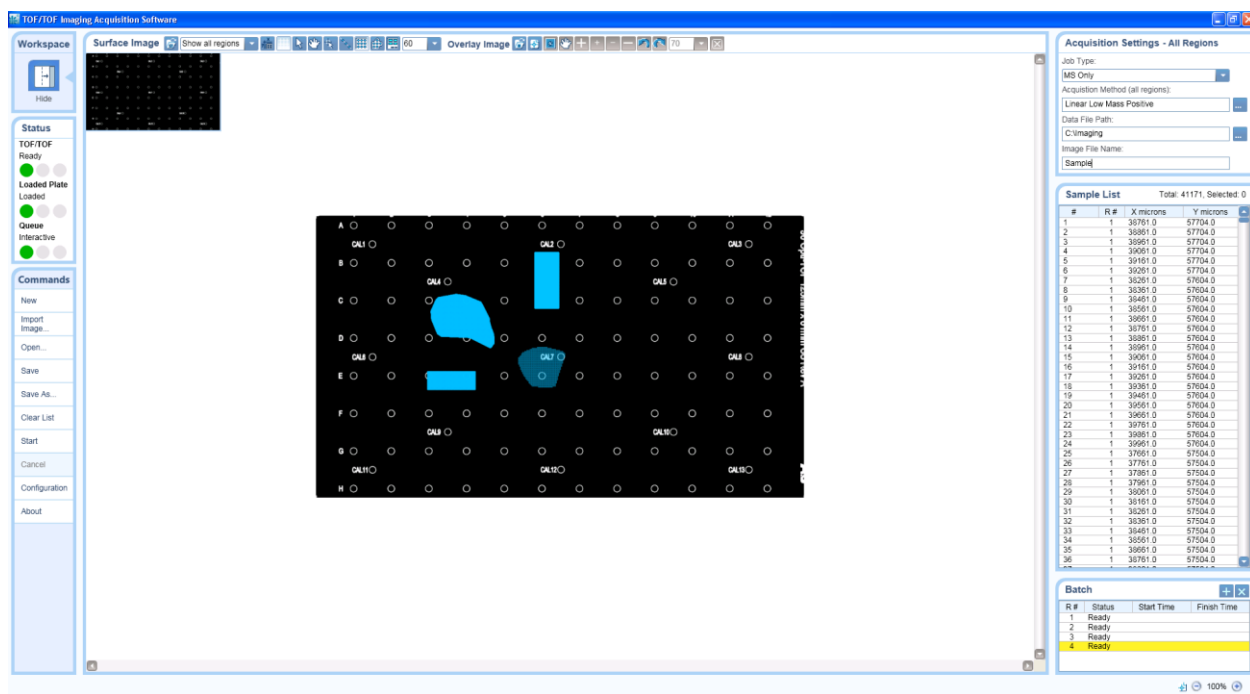


Figure 5. Image with Region 1 to 4 (“Show all regions” is shown).

14. Click **Start**. The TOF/TOF Image Acquisition Software and the TOF/TOF Series Explorer Software begin acquiring the selected spots on a region by region basis.

As part of the acquisition process, the software saves a copy of the Instrument Acquisition settings with the data as specified in the Data File Path. Additionally, the software saves a JPG image of the entire sample plate without the spots and separate JPG images for each region showing the spots designated for that region. These files may be useful for use in other processing applications.

**Note:** The TOF/TOF Image Acquisition Software acquires the data and then generates a set of files (\*.img, \*.hdr, \*.t2m) that can be read by the TissueView™ Software. The imaging data is stored in the Analyze 7.5 format.

## NOTES

**Status Panel:** The TOF/TOF Series Explorer Software must be open, the plate loaded, and the Queue in Interactive mode for the Start button to become enabled.

**Configuration:** Standard 384 and 96 well Opti-TOF 123 x 81 mm plate templates are provided with Upper Left/Upper Right and Lower Left/Lower Right XY coordinates that correspond to the engraved “+” fiducials. A User Defined plate template with configurable XY alignment coordinates is available.

**Configuration- Output Image Scale Factor:** \*.jpg files of the image and spot layouts for each Region are automatically generated when an acquisition is started. A higher output factor creates a file with greater resolution (and size).

**Acquisition Method:** Only fixed position and continuous motion methods can be used. The number of digitized points in the method must be less than 65535 due to limitations in the Analyze 7.5 file format.

**Add Spots:** Use the toolbar buttons to add individual spots, a line of spots, rectangular areas, or irregular area of spots. You can also manually enter XY coordinates for a rectangular area of spots using a toolbar button. The spacing between spots is selected from the list. We recommend that Snap to Grid On is used when adding a line of spots or rectangular/ irregular areas of spots.

**Zoom In/Out:** Use the buttons in the lower right status bar to Zoom In/Out or Reset the primary image.

**Overlay Image:** Use the buttons in the toolbar to add, position, and resize a secondary image if required.

**Selecting and Moving Spots:** Spots can be selected for moving to a new or different region by using the toolbar Select Spot button to highlight the spots of interest and then selecting Move Selected from the right-click menu.

**Selecting and Deleting Spots:** Spots can be selected for deletion by using the toolbar Select Spot button to highlight the spots of interest and then selecting Delete Selected from the right-click menu. Spots can also be deleted by highlighting rows in the Sample List and selecting Delete Selected from the right-click menu in the Sample List.

**Move to Position when Available:** Use this toolbar button and the Video Viewer in TOF/TOF™ Series Explorer™ Software to confirm the image alignment by clicking on the sample image in the TOF/TOF Imaging Acquisition Software and verifying the sample stage moves to the correct location. If the position is not correct, make sure that a plate alignment within the TOF/TOF Series Explorer Software was performed correctly, the plate type is selected in the TOF/TOF Imaging Acquisition software is correct, and so forth.

**Note:** The TOF/TOF™ Imaging Acquisition Software will move the sample stage in the instrument under certain conditions (e.g. the instrument is not in “batch mode” and the TOF/TOF Imaging Acquisition Software is not actively acquiring, and so forth).

## Known Issues and Limitations

1. Caution: The 4800 Imaging Tool and TOF/TOF™ Imaging Acquisition Software must not be used at the same time. The 4800 Imaging Tool operates independently of the TOF/TOF™ Series Explorer Software and the TOF/TOF™ Imaging Acquisition, using it simultaneously can lead to unexpected instrument control issues, erroneous behavior and results.
2. Caution: Using the TOF/TOF Series Explorer Software to setup or perform acquisitions while the TOF/TOF Imaging Acquisition Software is acquiring data will cause the imaging acquisition to stop and the imaging data may be unusable.
3. The Spot coordinates always align with a grid having a minimal grid size of 10 um by design. The grid is used for mapping each spot to a single pixel of the output image (necessary for the TissueView™ Software). Pixels not associated with the particular spot and in between spots within a region will be filled with an empty spectrum.
4. Selecting multiple areas of spots that are not in close proximity on the region may create a file that is too large for TissueView™ Software and the acquisition may not proceed. Reduce the file size by having the areas of interest spaced closer together or use multiple regions via the Create New Region functionality.

5. For a large number of spots - a delay may be observed when the user clicks Start and the laser resumes firing. For example – for 20000 spots - one shot is acquired and then *Creating Image files...* is shown in the status bar. It takes approx. 20 sec for the file creation to complete and then the laser resumes firing. If the user clicks Cancel during acquisition or *Creating Image files...* it may take 20 to 60 sec to complete the action. There is no indication to the user that the application is busy.
6. A continuous motion method for acquisition may yield undesirable results because the sample stage will always move towards the nearest spot location defined in the Spot Set Template that is used to define the plate layout. This is a limitation due to the TOF/TOF™ Series Explorer™ Software.
7. If the Output Scale Factor in the Configuration window is too high, the automatic generation of the \*.jpg files may take a long time. At higher scaling factors, the \*.jpg file may not be generated due to an Out of Memory condition and a warning message is shown. The actual data acquired is not affected and the acquisition of the regions will proceed. If the \*.jpg image file is needed, restart the TOF/TOF Imaging Acquisition Software and restart the acquisition. Alternatively, restart the acquisition using a lower Scaling Factor (see Configuration button).
8. Adding spots and selecting many spots (>250,000) for deletion might cause your computer to run out of memory and the application to stop responding. Other symptoms can include very slow performance or “Out of Memory” error messages. If any of these problems occur – consider increasing the spacing level to reduce the number of spots in the Sample List. Use Clear List in the Commands panel to remove all the spots from the image and Sample List.
9. Very slow performance or application not responding is observed when Zooming In from 1500% to 2500% with “Display spot numbers on image” checked in the Configuration window for a large number of spots. For example, for 20000 spots - Zooming In from 1500% to 2500% may take 60 seconds or longer. For a larger number of spots for example, 50,000, the application will stop responding. We recommend that users clear the “Display spot numbers on image” check box in the Configuration window when using a large number of spots.
10. Acquisition settings files (\*.IAS) from previous beta software versions are not compatible with the released V1.0 software.
11. Rotation of an Overlay Image is limited to small increments. Use another program such as Microsoft Paint or Microsoft Picture Manager to rotate the secondary image more quickly.
12. Rotating a large image file (> 5 MB) multiple times in the Alignment Wizard may result in Out of Memory errors.



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