



---

**Title: Digital Gel Imaging Using the Kodak 400 Image Station**

SOP Number: 22120

Revision Number: 04

Supersedes: Revision 03

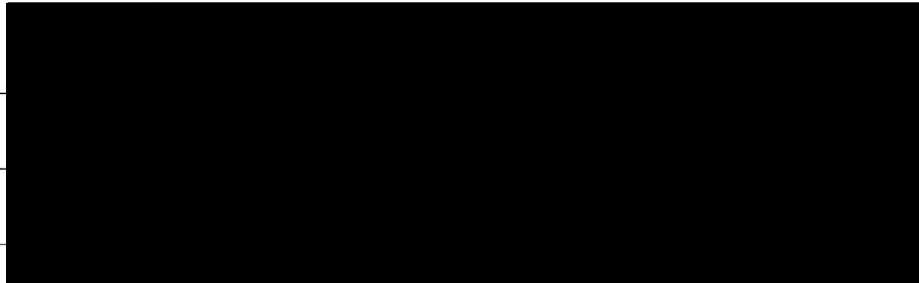
Effective Date: APR 28 2016

---

Originator/Date: \_\_\_\_\_

Approval/Date: \_\_\_\_\_

Approval/Date: \_\_\_\_\_



**Table of Contents**

- 1.0 Purpose
- 2.0 Scope
- 3.0 Authority and Responsibility
- 4.0 Abbreviations and Nomenclature
- 5.0 Reagents and Materials
- 6.0 Equipment
- 7.0 Procedure for Gel Imaging
- 8.0 Image Analysis Software Applications
- 9.0 Documentation
- 10.0 References and Related Documents
- 11.0 Attachments

**1.0 Purpose**

This Standard Operating Procedure (SOP) details the procedures, methods, and equipment settings for the digital capture and automated analysis of agarose and acrylamide gels using the NEN/Kodak 440CF Digital Imaging Station and the Kodak 1D Image Analysis Software (version 3.0.1 or later) by Biopharmaceutical Development Program (BOP) staff.

**2.0 Scope**

This procedure applies to Process Analytics/Quality Control (PA/QC), Production, and Development personnel in the BOP. The Kodak 440 Imager and this Standard Operating Procedure (SOP) is for use with external visible (Vis) and external or internal ultraviolet (UV) light

sources and a variety of gel formats including: agarose gel electrophoresis (AGE) of DNA and RNA, SDS polyacrylamide gel electrophoresis (SDS-PAGE) - including Novex gels, native PAGE, and other transparent non-gel media such as agar plates up to a maximum size of 20 cm X 25 cm. The Kodak Image Station is fitted with a variety of custom changeable filters that are manually selected based on the staining dye (if any) used for detection of nucleic acids or proteins. The Kodak 1D Image Analysis Software can be used to accurately and objectively size (in bp, Da, or mm units), quantitate (absolute or relative mass, O.D., or fluorescent intensity), and count gel lanes and bands with minimal human intervention and reduced potential for bias errors in gel analysis. Finally, the Image Analysis Software saves raw and quantitated images as well as data files in an electronic format, allowing for permanent network archival and future review (or re-analysis) of archived digital images at remote locations.

### 3.0 Authority and Responsibility

- 3.1 The Director, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.
- 3.2 PA/QC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).
- 3.3 PA/QC personnel are responsible for the performance of this procedure.
- 3.4 PA/QC is responsible for reviewing the data and documentation of the results of this procedure.
- 3.5 BQA is responsible for quality oversight of this procedure.

### 4.0 Abbreviations and Nomenclature

- 4.1 AGE – Agarose Gel Electrophoresis (Used for nucleic acid separations).
- 4.2 Band – A lane bound region in a gel created by a single (or small number) molecular species.
- 4.3 Band Pass – A type of optical filter that removes wavelengths of light outside a specified range.
- 4.4 CCD – Charged Coupled Device, the cooled electronic “film” of the Image Station.
- 4.5 Clasp – the plastic locking tab on the instrument cover, needed to seal out external light.
- 4.6 CW – Center Wavelength of light that a band pass optical filter will pass with high efficiency.
- 4.7 EtBr – Ethidium Bromide (a fluorescent staining dye of nucleic acids).
- 4.8 Filter – A coated glass optical lens that selectively modifies the light passing through.
- 4.9 Filter Wheel – Rotating mechanical wheel in the Image Station that holds 5 band pass filters.
- 4.10 f-stop – Measurement of the opening size of a light blocking diaphragm in a lens.
- 4.11 Lane – A feature of a gel defined by the location of a loaded well formed by a gel comb.
- 4.12 nm – nanometer, common measurement unit of light in wavelength distance.
- 4.13 PAGE – Polyacrylamide Gel Electrophoresis (Used primarily for proteins and small DNA).
- 4.14 Parfocal – A lens system able to maintain focus over a range of magnifications (zoom).
- 4.15 Pixel – Fundamental unit in a CCD digital camera, smallest unit of light detection.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract HHSN261200800001E.

- 4.16 Platen – Coated clear optical plate on top of the Image Station, the imaging window.
- 4.17 ROI – Region of Interest, used with non-gel based imaging and analysis.
- 4.18 S/N – Signal to Noise of a null image, Critical specification for CCD imaging of gels.
- 4.19 UV – Ultraviolet light.
- 4.20 UV lamps – Two internal UV fluorescent lamps that provide low intensity 300-400 nm light.
- 4.21 Vis – Visible light.
- 4.22 Zoom – The ability of a lens to change the image magnification of an object.

## 5.0 Reagents and Materials

**NOTE:** Never use metallic or abrasive materials (such as razors, tweezers, metal scoops, or abrasive cleaning solutions) on the optical platen.

No specific reagents or materials are required to use the Kodak 440CF and software; however, the following items will prove to be of use.

- 5.1 Plastic gel scoops for transporting gels to and from the platen.
- 5.2 Kimwipes to remove excess water from the platen surface.
- 5.3 Latex or Nitrile gloves to position the gel on the platen surface.
- 5.4 Non-abrasive, non-foaming ammonia based glass cleaner.
- 5.5 Compressed air/N<sub>2</sub> can for removing surface dust on the platen.

## 6.0 Equipment

- 6.1 Kodak Digital Science Image Station 440CF.
- 6.2 Kodak Optical Diffuser plate for taking images of unstained gels.
- 6.3 Kodak alignment and zoom transparency to verify lens settings.
- 6.4 Adapted external UV or optical light box for rapid image acquisition of faint images.
- 6.5 Appropriate band-pass optical filter (selected on the filter wheel).
- 6.6 Kodak 1D Image Analysis Software (version 3.0.1 or later).
- 6.7 Windows 98 or newer PC with USB and a parallel port with the Kodak software protection key.
- 6.8 VGA computer display at a minimum 800x600, 16-bit color resolution.
- 6.9 Network connection for data archival and retrieval.
- 6.10 Microsoft Excel 97 SR2 (or later version).
- 6.11 For further detailed requirements, see the Kodak Image Analysis Software Manual.

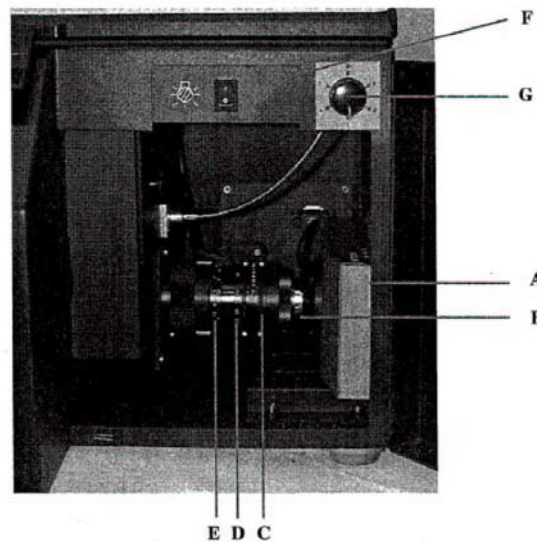
## 7.0 Procedure for Gel Imaging

- 7.1 General Instrument Setup
  - 7.1.1 Turn on the Kodak Image Station via the rear power switch. “1” indicates “ON.”
  - 7.1.2 Turn on the instrument IBM computer via the front panel switch, and allow for boot-up. Log on the network using your personal network ID and password.

This procedure is made available through federal funds from the National Cancer Institute, NIH, under contract HHSN261200800001E.

- 7.1.3 Open the Kodak 1D Image Analysis Software by double clicking the desktop icon. Once the Kodak software is on-screen, select the **CAPTURE TWAIN** box located in the upper left-hand corner to open the IS440CF Capture screen.
- 7.1.4 Open the instrument's top lid by unlocking the clasp and ensure the platen is clean and free of streaks or dust. Clean with an ammonia-based cleaner if necessary.
- 7.1.5 Open the instrument's front cover panel to expose the UV and filter switches, and the zoom lens. Ensure that the focus ring is set to the white dot.

Capture System Chamber



**NOTE:** The focus ring should never need adjustment and should not be changed. Set the f-stop ring to either 1.2 or 2.0 (largest openings) to allow the quickest image capture time. See the chart in 7.2.3 for recommended f-stop and zoom settings for various gel sizes.

## 7.2 Gel Placement and Alignment

- 7.2.1 Place the gel to be imaged on the platen with the well openings facing up (if applicable). Leave the top lid open to allow room light to enter the platen.
- 7.2.2 Select the **PREVIEW** box in the Kodak software Image Capture Settings dialog box and select **EXPOSE** to view the gel as it is moved into the desired alignment and the zoom is changed to accommodate the full size of the gel, but **Do Not** include the gel edges.

**NOTE:** For certain applications, only portions of a gel may be imaged for increased resolution.

- 7.2.3 Change the image zoom setting by rotating the zoom ring located between the focus and f-stop rings. The zoom can be adjusted from 12.5 mm to 75 mm (a 6X magnification). For recommended f-stop and zoom settings for various gel types and sizes, see the chart below.

GEL SIZE	UV REQUIRED?	F-STOP	ZOOM
X 14 cm	YES	1.2 or 2.0	21.9 mm
X 25 cm	YES	2.0	12.5 mm
Novex	NO	≥ 1.2	~ 40 mm

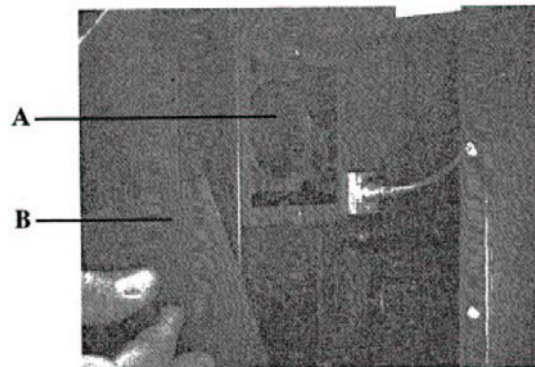
- 7.2.4 Once the gel is centered, the zoom and f-stop selected, prevent the gel from shifting during imaging by removing any excess water or buffer from the edges of the gel with a kimwipe.
- 7.2.5 Close the top lid and lock the clasp if the gel requires fluorescent (UV) illumination.
- 7.2.6 For gels requiring natural room illumination (such as Coomassie gels), cover the gel with the opaque Optical Diffuser Plate, silicone feet down, and leave the instrument lid open. Some adjustment (increase) of the f-stop may be required to increase the exposure time for the image capture, adjust the f-stop while in Preview mode.
- 7.2.7 Stop the Preview image captures by clicking **STOP** exposure and de-select the Preview check box.

7.3 Filter Selection for Fluorescent Dye Stained Gels

- 7.3.1 Rotate the FILTER SELECTION DIAL clockwise (located on the instrument front panel) to the appropriate filter position as indicated on the inside front cover panel and listed below.

POSITION	FILTER/DYE	Center Wavelength	>5% Trans Range	Omega ID #
0	SYBR Green I & II	517 nm	507 – 528 nm	517AF20
1	SYPRO Orange	575 nm	565 – 586 nm	610DF40
2	Fast Filter For all Gels	610 nm	500 – 800 nm	562.5DF75
3	Open (No filter) for natural illumination images			N/A
4	EtBr or SPYRO Ruby	650 nm	500 – >800 nm	500AGLP
5	SYBR Gold	532 nm	522 - 549 nm	532DF20

**NOTE:** When changing the filter position on the selector dial, ensure that the filter wheel is rotating properly by removing the magnetic filter access panel on the left side of the front access chamber. Occasionally, the filter wheel may have to be manually moved while the dial is being turned - Wear fresh Nitrile gloves (no powder) and do not touch the filter glass when manually rotating the filter wheel or removing filters for maintenance. Filters are labeled on the side for band pass wavelength and directionality. Once the proper filter is in place, remember to replace the magnetic filter access panel.



**A** *Filter Wheel* contains the filters for different detection methods. There are six spaces in the wheel. Position "0" should always be without a filter. The remaining five filters are standard 58mm filters. The filters can be changed if necessary.

**B** The *Filter Access Panel* provides access to the filter wheel and is light-tight. Remove only the door when changing a filter.

7.3.2 Turn on the UV lamps via the front panel switch (position "1") and close the front panel door.

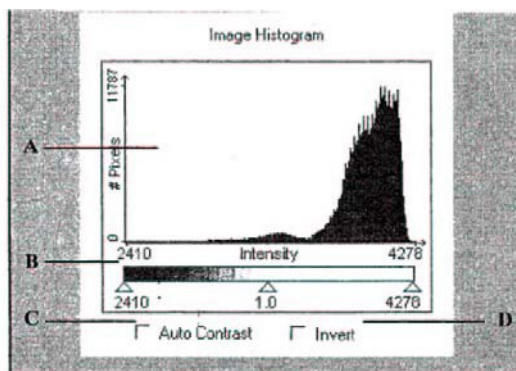
#### 7.4 Acquiring the High Resolution Gel Image

7.4.1 Using the **FILE/OPEN** command in the Kodak 1D software, open the Illumination Reference file in [REDACTED] that contains the appropriate f-stop (F) and zoom (Z) that is being used with UV illumination (i.e., UV-F1.2-Z21.9.bip). This file image will be used to correct the gel image for variations in the UV lamp intensity after the image has been captured. If the appropriate Illumination Reference file is not available, continue with 7.4.2 and contact the PA/QC Supervisor or appropriate development scientist for more information.

7.4.2 Using the capture number and exposure time software slider controls in the IS440CF Capture screen, set the number of exposures to  $\geq 6$  for UV illuminated gels and to  $\leq 6$  for naturally illuminated gels. Also, set the image exposure time at 2 minutes for UV illuminated gels, and at 0.075 to 0.1 seconds for naturally illuminated gels.

7.4.3 Click on **EXPOSE** in the IS440CF Capture screen to begin the image capture. An initial image will appear after the first exposure is complete. Wait until all exposures are complete unless the image is saturated or exceeds 5000 intensity levels as indicated in the IMAGE HISTOGRAM window.

Image Histogram Display and Slider Available in the IS440c Image Acquire Window



- A** The *Image Histogram* displays the range of signal of the image. The histogram can be used to determine the optimal exposure time so the image has the largest possible signal range. The numbers directly below the histogram indicate the range of gray levels for the current image. For multiple capture images, this range can be up to 65,536 signal levels. For single capture images, this range can be up to 4,096 signal levels.
- B** The *Contrast Slider* sets the black and white points and gamma for viewing the image on screen. (i.e. the closer the black and white points the higher the contrast of the image.)
- C** *Auto Contrast* chooses the optimal white and black points and maximizes the on-screen appearance of the image.
- D** The *Invert* checkbox inverts the image from white signals on a black background to black signals on a white background.

7.4.4 If the post-capture image is too dark (low intensity level) increase the exposure time accordingly. If the post-capture image is too bright (high image intensity) or saturated first, reduce the exposure time, then (if necessary) for naturally illuminated gels, increase the f-stop to block more light from reaching the camera.

7.4.5 The IMAGE HISTOGRAM window should display a maximum signal level greater than 4000 on the lower right-hand corner. Following exposure, the histogram should be black; a red histogram indicates that the digital image is saturated due to excessive exposure. **Do not use, analyze, or save an image that exhibits saturation in the area of interest or region to be analyzed - recapture the image as per 7.4.4.**

**NOTE:** Ensure that only the gel is imaged as gel edges tend to increase the image intensity without providing useable data.

7.4.6 Note the maximum image intensity level for an image that is to be saved and analyzed. This information will be recorded in 7.5.3.

## 7.5 Correcting and Saving the Gel Image for Analysis

7.5.1 Click on **SUBMIT** to save the desired gel image. The Image Field Correction Window will appear. Set the indicated parameters using the digital sliders according to the Zoom, f-stop, filter ID actually used. Indicate the light source (UV/Fluorescence or None/Luminescence if using natural light), and indicate the appropriate open Illumination Reference file image if using UV illumination. Do not apply the <LENS CORRECTION> option.

- 7.5.2 Save the illumination corrected gel image by selecting [REDACTED]. For PA/QC test request images, the file name will be created according to the following nomenclature.

PA/QC<Request Number>.bip

For example: QC010000.bip

If multiple images are made of the same gel or multiple gels are run for the same test, number each image consecutively following the product lot number.

For example: QC010000\_3.bip

Once the image file name and the desired file path are input, select **OK** to save the file. The .bip file extension is automatically made; do **not** select the .tif extension unless exporting the file to a non-Kodak application.

- 7.5.3 The File Properties dialog box will open. Enter the following information to allow database searches and retrieval of the saved image.
- 7.5.3.1 Operator Name: Name(s) of the operator who performed the QC assay and who took the gel image.
  - 7.5.3.2 Date: Input the Month/Day/Year (e.g., 01/01/2002) for the gel run and image capture.
  - 7.5.3.3 Project: PA/QC request number, PA/QC request date, and requested test(s).
  - 7.5.3.4 Samples: The test sample name(s) and lot number(s).
  - 7.5.3.5 Comments: Include the gel lane designations with sample IDs, lots, volumes and mass loaded. Also, include any other comments deemed appropriate.
- 7.5.4 Select **OK** to close the File Properties dialog box and save the image file. Close any open Illumination Reference files before proceeding to image analysis.
- 7.5.5 Turn off the Image Station UV lamps and thoroughly clean the platen surface after the gel image is saved.
- 7.5.6 If the gel is to be discarded, place the gel in medical waste for incineration.

## 8.0 Image Analysis Software Applications

- 8.1 Saved image files may be analyzed immediately following step 7.5.4 or they may be analyzed remotely (if the .bip image file is saved to a network drive) at a later time using the Kodak 1D software.
- 8.2 To open a saved file for analysis, select **File/Open** and choose the path and file name of the desired file. Enlarge the opened file to full-screen for analysis. The gel image may be enlarged or reduced as desired using the magnification software slider at the bottom of the image.
- 8.3 Invert the image (black bands on a white background) and adjust the image contrast by selecting **Show/Image Display** from the top menu bar. The IMAGE DISPLAY window will open. Select **Display/Invert** to invert the image.



- 8.4 While the IMAGE DISPLAY window is open, select the AUTO button at the bottom right-hand corner to automatically adjust the contrast for optimal visual resolution. Close the IMAGE DISPLAY window.
- NOTE:** It is not recommended that the Min/Max/Gamma contrast sliders be manually adjusted; the AUTO contrast feature generally provides the best visual contrast.
- 8.5 Gel images may be flipped or rotated prior to analysis using the Edit/Flip and **Edit/Rotate** menus. Finer gradations of gel image rotation, if necessary for lane and band analysis, are achieved by selecting the **ROTATION** button on the left-hand menu bar (third button from bottom) and setting the mouse cursor at the top of a gel lane near the center of the gel. Continue to hold down the left mouse button while moving the cursor down the center of the selected lane. Release the left mouse button when the cursor has reached the bottom of the lane. A message "Processing Pixels" will be displayed and the gel image should re-align correctly.
- 8.6 Gel lanes and bands are automatically found via the 1D software by first selecting the **SELECT IMAGE** button on the left-hand menu bar (center button). Place the mouse cursor over the upper left-hand region of the gel to be analyzed (just below the wells) and while holding the left mouse button down, drag the cursor across the gel to the desired lower right-hand corner. Release the mouse button to show the selected gel area. If necessary, reselect the analysis area by removing the unwanted selection (left click anywhere off the area) and reselecting. Once the analysis area is created and boxed, deselect the **SELECT IMAGE** button by selecting the normal **SELECT** (arrow) button on the left-hand menu.
- 8.7 Find gel lanes in the boxed area by clicking on the LANES/Find Lanes tab located at the lower left-hand corner of the image field. The boxed area should show vertical blue lines corresponding to the average centers of the gel lanes.
- 8.7.1 If necessary, the gel lanes may be adjusted by clicking on **Adjust Lanes** in the **LANES** tab. The number, positions (move left or right), and the effects of gel smiling can be corrected for using automatic and manual lane adjustments on the **Adjust Lanes** window.
- 8.7.2 Changing the number of lanes - An incorrect number of lanes may be corrected by changing the lane number in the **Create Lanes** dialog box. After inputting the correct lane number, click on **Find Again**.
- 8.7.3 "Smiling." Correcting for gel distortion, i.e., gels can be corrected by selecting the appropriately shaped **Lanes** button.
- 8.7.4 Moving the position of lanes - The location of a gel lane may be adjusted by moving the mouse cursor over the desired lane, left clicking on the lane, and moving the lane in either direction to its desired location.
- 8.7.5 Changing the software lane sensitivity - Undetected lanes may occur due to the relatively low signal intensity of the bands in the lane. The software sensitivity is changed by moving the **Sensitivity** slider, followed by clicking on the **Find Again** button.
- 8.7.6 Wherever possible, use **Automatic** adjustment followed by clicking **Find Again** on the **Adjust Lanes** window. Once any adjustments are complete and all desired lanes are found, close the **Adjust Lanes** window.
- 8.7.7 Once satisfied with any lane adjustments, **Close** the **Adjust Lanes** window.

- 8.8 Find gel bands along the highlighted lanes by clicking on the **Find Bands** button in the **Lanes** tab. Bands found by the software will be indicated as numbered blue “ball-and-line” markers located over the image band. The “ball” portion of the band marker is numbered to indicate the relative position of that band in the lane. The top-most band in a lane is labeled as number “1.” On most gels the 1D software will label a few gel artifacts as bands and will not designate actual bands as such. These problems should be corrected before proceeding with band fitting.
- 8.8.1 Changing the software band sensitivity - If many more or less bands than expected are found by the software algorithm, first change the software sensitivity by clicking on **Adjust Bands** to open the **Adjust Bands** window. Change the sensitivity by moving the **Band Sensitivity** slider - the band number will immediately change on the image. Once the optimal sensitivity and band number are reached, Close the window.
- 8.8.2 Removing undesired bands - Bands that are not necessary to the image analysis (such as ladder bands well beyond the test sample bands) or bands that represent miscalled gel artifacts may be removed from the analysis by left clicking on the band and pressing the **Delete** key. The highlighted band will turn red and show the band edge limits as a red box before deletion.
- 8.8.3 Adding new bands to the analysis - If necessary, new gel bands may be created (where the software did not find a band) by:
- Selecting (highlighting) the lane in which the band will reside, and changing the left-hand menu screen to the **Lane Profile View** by selecting the **Blue Arrow** button at the bottom of the left-hand menu. This view allows easy placement of new bands due to the lane intensity histogram it displays.
  - Selecting the **Add Bands** tool on the left-hand menu bar (bottom button) changes the mouse cursor into a band marker shape. Using the **Lane Profile View** and the gel image, place the cursor over the most intense portion of the desired band area.
  - Left click the mouse to set the band on the image. If necessary, the band width and band center location may be adjusted on the **Lane Profile View** using the red band tabs.
- NOTE:** Do not overlap the new band position or band width with other bands.
- Continue for all other desired but unfound bands. Select each lane in turn for the **Lane Profile View**. The **Lane Profile View** can be returned to the menu screen by clicking on the **Blue Arrow** button.
- 8.8.4 Moving the position of bands for analysis - If necessary, band positions may be moved along a lane by using the **Lane Profile View** as outlined in 8.8.3 above or directly by left clicking on the band marker and dragging the band to the new location.
- NOTE:** Do not overlap the new band position or band width with other bands.
- 8.8.5 Adjusting the size of bands - If necessary, band widths (e.g., broad to narrow along the lane axis) may need to be adjusted by using the **Lane Profile View** as outlined in 8.8.3 above or directly by left clicking on the band marker and dragging the band

box outline to the desired new positions. Ideally, a band width should encompass the entire band intensity peak without overlapping the adjacent bands; if this is not possible, narrow the band width focused on the band intensity peak.

**NOTE:** Do not overlap the new band position or the band width with other bands.

- 8.9 If necessary, manually fit (analyze) the gel bands by clicking on the Fit Bands menu button. A Fitting **Bands** window will appear to indicate the progress of the analysis. The software will encounter quite often one or more problem bands during the analysis - resulting in an incomplete fit warning window. The problem bands will be highlighted in red by the software, and a new **Non-Converging Bands** window will appear. Usually, the problems are due to band overlap or excessive smearing. There are several ways to continue the fit analysis.
- 8.9.1 The simplest course of action is to simply delete the affected bands if allowed by the required PA/QC test.
- 8.9.2 Alternatively, the affected band may be adjusted for band width. Also review the adjacent bands in the lane to ensure that no overlap has or will occur.
- 8.9.3 If adjusting the band width does not result in a successful fit, the band will have to be deleted.
- 8.9.4 Once a band is adjusted for fit or deleted, select **Next Band** in the **Non-Converging Bands** window. Once all bands have been adjusted, click on **Fit Bands** in the **Non-Converging Bands** window. Usually, a few new bands are found as "non-converging;" continue with the manual sizing and/or deleting process until all bands are fit (Non-converging bands are equal to 0) and close the **Non-Converging Bands** window.
- 8.9.5 Alternatively, the non-fitting bands may be left in the analysis; however, the data obtained from the non-convergent bands should be noted and is automatically highlighted by the software.
- 8.10 Label and identify each lane on the image by right clicking on each lane and selecting **Lane Information** from the pop-up menu. A **Lane Information** dialog box will appear. Enter the requested data for each marked lane of the image (using the dialog box arrow buttons or directly selecting each lane) as follows.
- 8.10.1 **Lane Type** pull-down menu - choose whether each lane contains an **Experimental** (test sample), **Standard** (size or mass ladder, etc.), or is **Inactive** and not used.
- 8.10.2 **Lane Name** - for **Experimental** lanes input the desired name of the lane based on the sample or standard loaded in the lane. Always use the QC request number and loaded mass.
- 8.10.3 **Lane Name** - for **Standard** lanes select one of the pre-loaded standards, modify a pre-loaded standard (this is often necessary since not every band of a standard is usually used), or create a new standard from the **Standards** window. Click **Select** to formally assign a chosen Standard to the lane.
- 8.10.4 Modify existing standards to reflect the bands fitted in the gel image by selecting **Duplicate** from the **Standards** window, and renaming the standard in the **Edit Standards** window. Delete the bands listed in the **Edit Standards** window that are not to be used (or have not been fitted) in the gel image. Once editing is complete,

- click **OK**. In the **Standards** window, select the re-named and newly edited standard.
- 8.10.5 **Total Mass in Lane** - for **Standard** lanes, input the mass loaded in the lane and select the unit of mass measure from the pull-down menu.
- 8.10.6 Once all boxed lanes are identified, click **OK** in the **Lane Information** window.
- 8.11 View the lane analysis data following band fitting (Section 8.9) by selecting **Show/Lanes-Bands/Lane Analysis Data** from the top menu.
- 8.11.1 The initial **Lane Analysis Data** window is in a spreadsheet format. The information displayed in the window can be selected using the window's **Lanes** and **Display** menus.
- 8.11.2 Required display data for gels are: **Molecular Weight** (and/or **Mass**), **Net Intensity**, **Sum Intensity**, and **Background Intensity**.
- 8.11.3 Only select for display the lanes and analysis data that are necessary for the PA/QC test requirements.
- 8.11.4 Data shown in red is extrapolated from the nearest standard point and may not be accurate.
- 8.11.5 **Lane Analysis Data** and raw intensity **Histogram Data** may be exported to another program such as Microsoft Excel or Origin using the **File/Export Data** menu bar.
- 8.12 Annotate the gel image by selecting **Annotations, Show Annotations** from the main menu bar. The annotation screen and left-hand tool bar allows the addition of configurable text and pointers to the gel image.
- 8.12.1 Move the gel image in the annotation page preview window to the best location for adding text to indicate gel lane numbers, contents and an image header by selecting the "**arrow**" tool and left clicking on the gel image.
- 8.12.2 Select the "**A**" (text) tool and move the mouse cursor to the desired left-hand corner or the region to contain text, left click the mouse to open the **Text Options** dialog box.
- 8.12.3 In the Text Options dialog box, type the desired text and select the desired **Font** type, **Size** and **Style**. Also, select the desired text **Alignment** within the text region - the default is left-hand aligned. Finally, select the desired text **Rotation** or angle - typically an image header is rotated **0 degrees** (horizontal text) while lane numbers and lane identifications are rotated **90 degrees** (vertical text) or **45 degrees**.
- 8.12.4 Pointers and background text boxes may be added (but are typically not necessary) by selecting their respective tools on the left hand of the screen.
- 8.12.5 Once the text, font, and alignment options are complete, select **Set** in the Text Options dialog box. The resulting text on the gel image may be moved or adjusted by selecting the **Arrow** tool and dragging the text box as needed. Insert/change additional text or change the text options by double clicking the text box once the **Arrow** tool is selected.
- 8.12.6 New text to be placed in a different location on the annotated image can be added by repeating 8.12.2 through 8.12.5.

- 8.12.7 Image headers must contain the following information: The QC request number(s), sample name(s), sample lot number(s), assay type(s) [for example: restriction analysis or percent scDNA], the operator's name, and the date the annotations were made.
- 8.12.8 Lane designations must refer to the same lane numbers and descriptions as indicated in the **File Information** and **Lane Info** menus (see 8.10). The lane number annotation must appear directly above or below the respective well. The annotated image should also contain abbreviated lane descriptions either directly above the well/lane number or separately below the text header.
- 8.12.9 Once annotations are complete, save the file using the **File/Save** menu option. The annotations window may be closed by selecting **Annotations/Hide Annotations** from the main menu.
- NOTE:** The annotated gel image will print as a separate page from the analyzed and raw gel images. The annotated image cannot be exported to another image file format.
- 8.13 Print the gel image, file information, analysis spreadsheet and the PA/QC report by selecting the **File/Print** menu option. A **Print** selection window will appear.
- 8.13.1 Ensure the desired printer is selected for the computer.
- 8.13.2 Select the number of copies desired; only one is required.
- 8.13.3 Select the following components for printing from the **Print** window:
- **Image**
  - **File Information**
  - **Lane Analysis**
  - **Annotations**
- 8.13.4 Click on **Lane Layout** to open the **Lane Analysis Layout** window. Change the **Print Format** to **One Page Summary (w/Image)**, select the critical **Variable** required for the PA/QC test (e.g., **Molecular Weight** for a sizing assay), and select **All Lanes** in the **Print Analysis** dialog box. Select **OK** to return to the **Print** window.
- 8.13.5 After the initial print, also print the complete **Lane Analysis** data by selecting **Print Break Lane Data** in the **Vertical Axis** from the **Lane Analysis Layout** window. Select **OK** to return to the **Print** window.
- 8.13.6 Select **Print** in the **Print** window to initiate printing.
- 8.14 Label all printouts with the PA/QC request number, operator name(s), test sample descriptions, and lot numbers, date and signature of operator on each page. Also, indicate the part and lot numbers of the standards used. Finally, indicate this and all relevant SOP numbers along with their effective revision dates on the cover page of the report.
- 8.15 If the final PA/QC Pass/Fail analysis is performed using exported **Lane Analysis Data** with third-party software, include the relevant labeled (per 8.14) spreadsheet printouts in the PA/QC report.

## 9.0 Documentation

- 9.1 Record the use of the Kodak gel imager in the instrumentation logbook; include the QC request number(s), date of use, operator name, and the test type(s) performed.
- 9.2 Final QC reports using the Kodak imager should include the following minimum information:
  - A raw (unedited/un-analyzed) gel image.
  - The file image/lane information summary.
  - The image capture conditions information.
  - The lane analysis page(s) if performed.
  - Additional data from second party analysis software as necessary.
  - Annotated gel image indicating lane contents and sample information.

## 10.0 References and Related Documents

- 10.1 Kodak Image Station 440CF Users Manual, Eastman Kodak Company, 1998.
- 10.2 Kodak 1D Image Analysis Software Users Guide, Eastman Kodak Company, 2000.
- 10.3 Kodak 1D Image Analysis Software web site: <http://www.kodak.com/go/1D3>.
- 10.4 Omega Optical Filter specification files, PA and BQA.
- 10.5 Omega Optical web site: <http://www.omegafilters.com/prod/bandpass.html>.

## 11.0 Attachments

- 11.1 Attachment 1 Form 22120-01, Rev 04 MEF Equipment Logbook

