

Viewing and editing of Typhoon scanner images from 1D, 2D, and DiGE experiments at CIAN

Note: This document summarizes some background information and short protocols for image manipulation of Typhoon scans, excluding image analysis. For detailed instructions refer to the user manuals of the software cited.

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I. Background on images acquired on the Typhoon scanner

- **Typhoon Scanner Control** generates .gel files, and one .ds file per multichannel stack
- color depth is 16-bit greyscale
- resolution depends on scanner settings (pixel size), eg 100 μ m pixel size = 10 pixels per mm of original = 254 pixels per inch
- Scanner Control window shows scan progress, with overexposed pixels in red
[Note: this image is automatically adjusted to (what the computer thinks is) optimal contrast and brightness for screen display, so it doesn't tell you anything about the pixel intensities of your scan]
- resulting image has to be opened in downstream application program like **ImageQuant** or **QuantityOne** for intensity evaluation, editing, quantitation, export into other formats

II. Screen shots for quick reports

If you just want a quick representation of what you see on the computer screen, you can use screen shots. Note that the resolution is lower than with the true file export options.

- arrange items on screen to your liking
- press "Print Scrn" button (in upper row of keyboard buttons) to put image of entire screen into clipboard; use "Alt+Print Scrn" to capture active window only
- paste image into **PowerPoint** or **Paint** document, or open clip board in **ImageJ** with "File/New/System Clipboard"

III. Export .gel files: single-channel gel images into presentation formats

Note: Even if you don't know whether you might want to come back and do (quantitative) analysis of the image, always store a copy of the original .gel file, because any other format loses some information.

- open .gel file in **ImageQuant** (any module)
- use "edit image" tool
- "save as" .tif file
[Note: this is 16-bit TIFF, useful for analysis but maybe not for presentation]

or:

- open .gel file in **QuantityOne**: "File/Open", make sure to enable "all files"
- "File/Export to TIFF image", or "File/Export to JPEG image" as desired
[Note: useful option of exporting to TIFF for analysis, or for presentation; allows to reduce bit depth]

or:

- open .gel file in **ImageJ**: "File/Open", or drop file icon on ImageJ icon
- [optional, but recommended for most presentations: change bit depth in "Image/Type" pull-down from 16 to 8-bit]
- "File/Save as", several different file formats offered

IV. Export .gel files: multichannel image overlays

Note: The .ds file can only be used in **ImageQuant** to open an image stack. In other programs, open the individual .gel files.

- open .ds file stack in ImageQuant
- view as overlay, activating relevant channels
- adjust contrast, brightness to show features of interest
- to zoom to area of interest, click on magnifying glass in bottom right corner of image screen, then drag across area of interest
- “File/Export to Clipboard” to copy to clipboard, or “File/Export to File” to create a Bitmap-type image file (.bmp file)

If you prefer another output file type, you could do screen shots, or open the .bmp file (or clipboard content) in **ImageJ** and save as something else.

Or you can create your own overlay in **ImageJ**

- open all the .gel files of your multicolor gel in **ImageJ**, ideally cropped to area of interest
- [optional: “Process/Enhance Contrast”]
- lower the bit depth: “Image/Type...” -> 8-bit
- create overlay: “Image/Color/RGB merge...”; select which image will be which color (typically, Cy2 = blue, Cy3 = green, Cy5 = red)

V. ImageQuant post-analysis export options at a glance

The images and tables created by an analysis in **ImageQuant** can be exported in multiple ways for use in other programs. This is a description of the different export options in the “File” menu. What is being exported depends on which of the three windows is currently active (image, analysis, measurements window).

“File/Export...”

“...to clipboard”

image, analysis:	RGB TIFF of window content as displayed
measurements:	table values as displayed

“...to file”

image, analysis:	bitmap (.bmp) of window content
measurements:	table in text format

“...to Excel”

image:	pixel value table of image, opens in Excel
measurements:	table opens in Excel

“...lane profile to clipboard”

analysis:	tab-separated values of lane profile [for pasting into spreadsheet to plot profile]
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For export of only the gel image (without any overlays) to greyscale TIFF, use the “Edit Image” tool as described in section III.

VI. Export of DeCyder analysis images

- in **DeCyder**, images are copied and pasted through the clipboard
- the general “edit/copy” command refers to the object or image in the currently active part of the window (where you last clicked); see also the status indicator on the bottom right edge of the frame (“focus: ...”)
- appearance can be adjusted before copying using the “Properties” dialog box, eg for color of spot boundaries, size of 3D peak area, font size of annotations
- even if spot outlines are not displayed, spot centers are always marked; for gel images without any overlays use **ImageQuant** or **QuantityOne** exports of .gel files
- gel images with or without annotations or spot outlines, and 3D peaks should be pasted into **Paint** or **ImageJ**, then saved as .tif for subsequent processing
[Note: gel images and 3D peaks cut and pasted directly into **PowerPoint** result in presentations that don't work on Mac computers]
- scatter plots, heat maps, PCA graphs etc pasted into **PowerPoint** can be “ungrouped” multiple times until all elements are recognized as individual objects, to be able to edit and adjust line width, font size, colors, etc
[if you are totally unhappy with the graphical appearance of plots: copy into your graphics program of choice, trace image with drawing elements from that program in a separate layer, delete original object – but don't complain to me if someone tells you that is fraudulent...]
- DeCyder module “XML toolbox” allows download of data from all modules except EDA; can be useful for numerical values, but copy-and-paste (for example into **Excel** spreadsheets) also works for the tables in the DeCyder windows and might be more straightforward

VII. Gel images and spot identities for robotic spot picking from DiGE gels

GenomeQuebec needs two images of the pick gel, both showing the stain that is used for picking:

1. an image of the entire gel with the pick spots indicated as a print-out
 - import and match stained pick gel image to DiGE project images in **DeCyder**, making sure that pick proteins are matched
 - show only pick proteins, add annotations with Master spot number
 - copy gel image with pick protein signatures and annotations to clipboard
 - open **ImageJ**; “File/New/System Clipboard” opens image
 - to crop, drag rectangle around gel, “Image/Crop”
 - print on laser printer in office; page setup: center on page, turn 90°
 - save as file type of your choice
2. a TIFF file (8-bit greyscale) of the entire gel without any overlays for triangulation on the picking robot
 - on Typhoon computer, open cropped .gel image file of pick gel in **ImageJ**, “Image/Type...” set to 8-bit, “File/Save as” .tif; take along on USB stick
 - or export .gel image file from **DeCyder** organizer window, open in **ImageJ** and change as above

VIII. Glossary of file types and relevant software

file types:

- .gel image generated on Typhoon scanner; proprietary (originally from Molecular Dynamics) variation of 16-bit TIFF image, including some scanning parameter information and calibration tables; for any image analysis, keep this format; can only be read and edited by special programs for gel imaging
- .ds data set file: small text file describing the link between individual .gel image files generated from the same scan, ie the Cy2, Cy3, and Cy5 images of a 3-color DiGE gel
- .tif raster image file, can come in different variations, e.g. different bit depths, but can generally be read by most image manipulation programs
- .jpg compressed image file format; useful for some forms of presentations, but not for analysis; can be read by most image manipulation programs
- .bmp Windows bitmap file format, uncompressed; can be read by most image manipulation programs

software:

- Typhoon Scanner Control (GE) – one registered copy on Typhoon PC
controls Typhoon scanner function; output are .gel files, and, for multi-channel images, .ds files
- ImageQuant TL (GE) – one registered copy on Typhoon PC
1D gel image analysis software; reads .gel/.ds files and .tif files
- DeCyder 2D (GE) – one license, hosted on DeCyder PC, client on Typhoon PC
2D gel image analysis software, specialized for DiGE analysis
- QuantityOne (BioRad) – basic version free download, PC or Mac
1D gel image analysis software; reads .tif and .gel files (not .ds)
- ImageJ (NIH) – free download (rsb.info.nih.gov/ij/), PC or Mac
general image viewing and editing; reads almost any image file format
- Microsoft Powerpoint – on all CIAN computers
presentation software, might not like all flavours of TIFF images
- Microsoft Paint – on all PCs as an Accessory program
basic raster image manipulation
- Microsoft Excel – on all CIAN computers
spreadsheet manipulation software