DNA Analysis by Native Polyacrylamide Gel Electrophoresis and Infrared Fluorescence Imaging Version 2

Michael Van Dyke,James Cox

Abstract

Short DNA probes, for example, those used in characterizing protein-DNA complexes, have historically been radiolabeled with ^{32}P to allow their detection and quantitation following native polyacrylamide gel electrophoresis (PAGE). For reasons of economy or safety, alternative means of DNA detection need to be used. Described here is the resolution of 5' IRD700 and IRD800 end-labeled DNAs by native PAGE and their visualization and quantitation by IR fluorescence imaging.

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Guidelines

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Before start

Stock reagents used:

5× Tris-borate EDTA (TBE) buffer

27 g Tris base

13.8 g Boric Acid

1.86 g EDTA•Na_2•2 H_2O

Weigh separately. Dissolve with stirring in 400 mL deionized water. Dilute to 500 mL final. Store at 4 ºC in a tightly capped 500 mL glass bottle. Routinely stable for over 2 months. The appearance of an accumulating white precipitate indicates that TBE buffer is no longer suitable for use.

40% (19:1) Acrylamide

19 g acrylamide

1 g bisacrylamide

Weigh separately. Dissolve with stirring in 40 mL deionized water. Dilute to 50 mL final. Store at 4 ºC in a tightly capped 50 mL plastic centrifuge tube. Routinely stable for over 2 months.

10% Ammonium persulfate

Weigh approximately 100 mg ammonium persulfate in a 1.5 mL microcentrifuge tube. Add 9× measured weight in deionized water (approx. 900 µL). Vortex to dissolve. Store at 4 ºC, tightly capped. Routinely stable for up to 1 month.

6× Native Orange Loading Dye

2 g glucose

90 mg Orange G

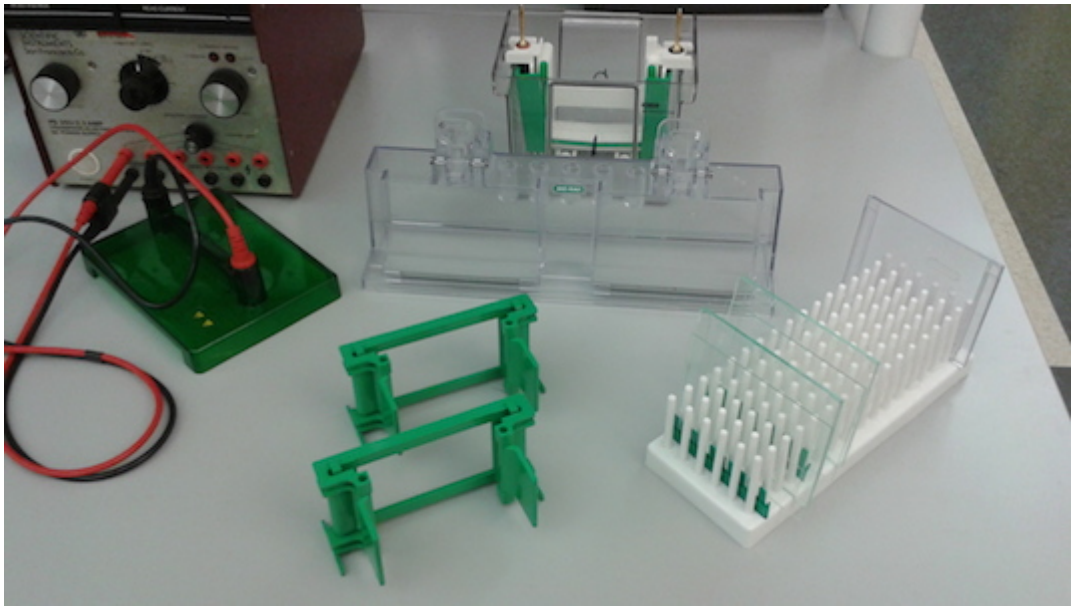
Weigh separately. Dissolve with stirring in 8 mL deionized water. Dilute to 10 mL final. Aliquot into ten 1.5 mL microcentrifuge tubes. Store at –20 ºC. Stable indefinitely. Thaw and vortex to mix thoroughly before use.

Protocol

Handcast native polyacrylamide gels

Step 1.

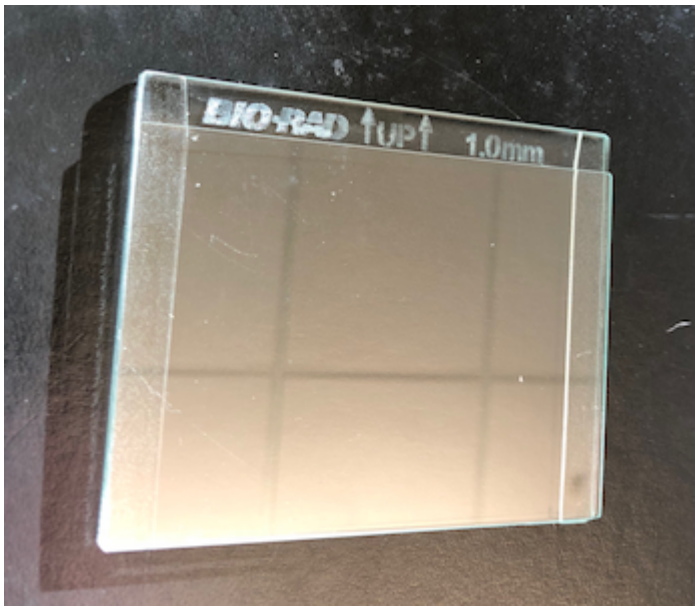
We routinely use the Bio-Rad Mini-PROTEAN® vertical electrophoresis system to cast and run 10-well 1.0 mm-thick 9.5%:0.5% polyacrylamide:bisacrylamide minigels in ½X TBE buffer [44.5 mM Tris base, 44.5 mM boric acid, 1 mM EDTA]. Needed materials: Bio-Rad casting stand, two casting frames, two short plates, two 1.0 mm spacer plates, two 1.0 mm 10-well combs, and one Mini-PROTEAN® vertical electrophoresis cell with buffer dam. Needed reagents: 5x TBE buffer, 38:2 acrylamide:bisacrylamide, 10% ammonium persulfate (APS), and tetramethylenediamine (TEMED), all stored at 4 ºC.



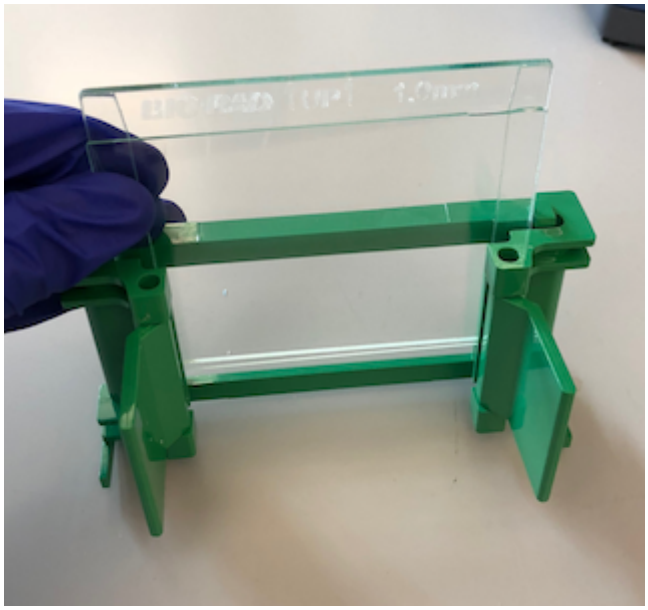
Handcast native polyacrylamide gels

Step 2.

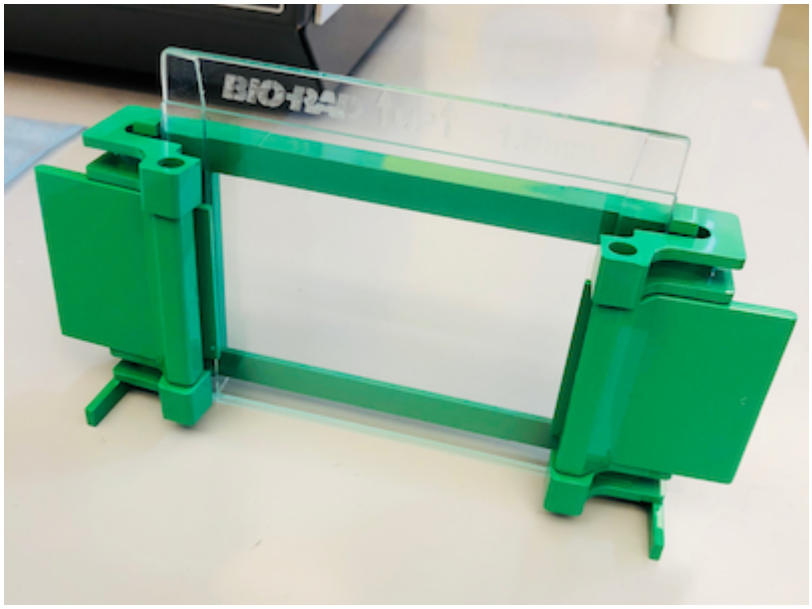
To assemble the gel casting apparatus, place a short plate on top of a spacer plate. Note that there is an internal space between the two and the writing on the spacer plate is exposed at the top of the sandwich.



Slide the two plates into an unlocked casting frame (clamps forward) from the top while on a level surface, making sure the short plate is oriented to the front of the frame. With the plates and frame flush with the level surface, lock the assembly by rotating the clamps 90º from their forward to their outward position. Check by hand to ensure both plates and frame are flush at the bottom. Repeat procedure if not.



Place the entire assembly onto a Bio-Rad casting frame with the short plate forward and the casting frame seated with its base back touching the casting stand. Lock the plate/casting stand in place by engaging the spring-loaded lever on the top of the casting stand and applying pressure to the top of the spacer plate with the short plate oriented forward. Now it is ready for gel casting.

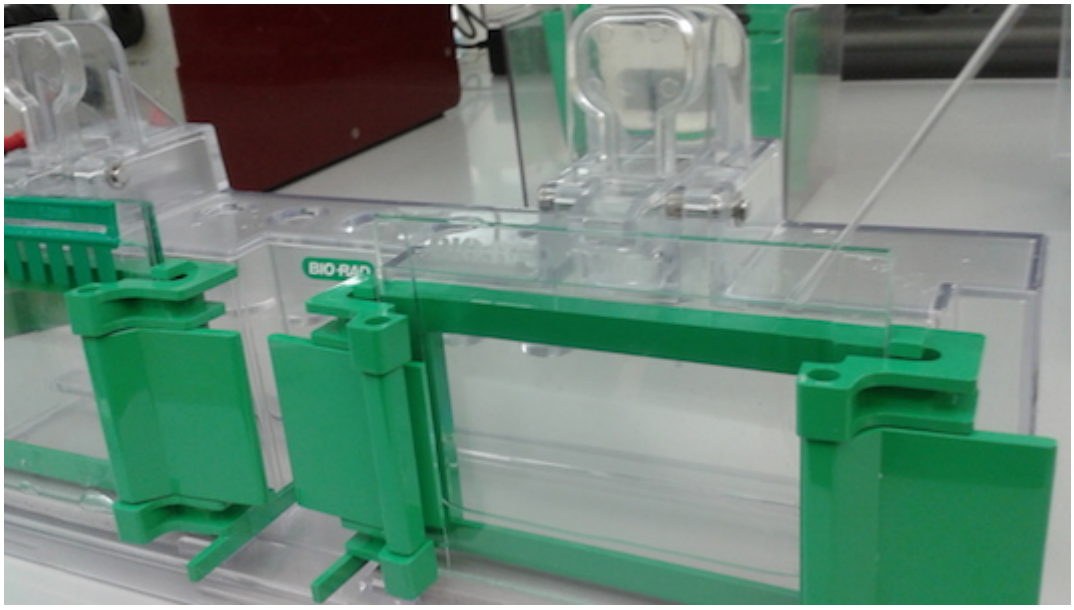


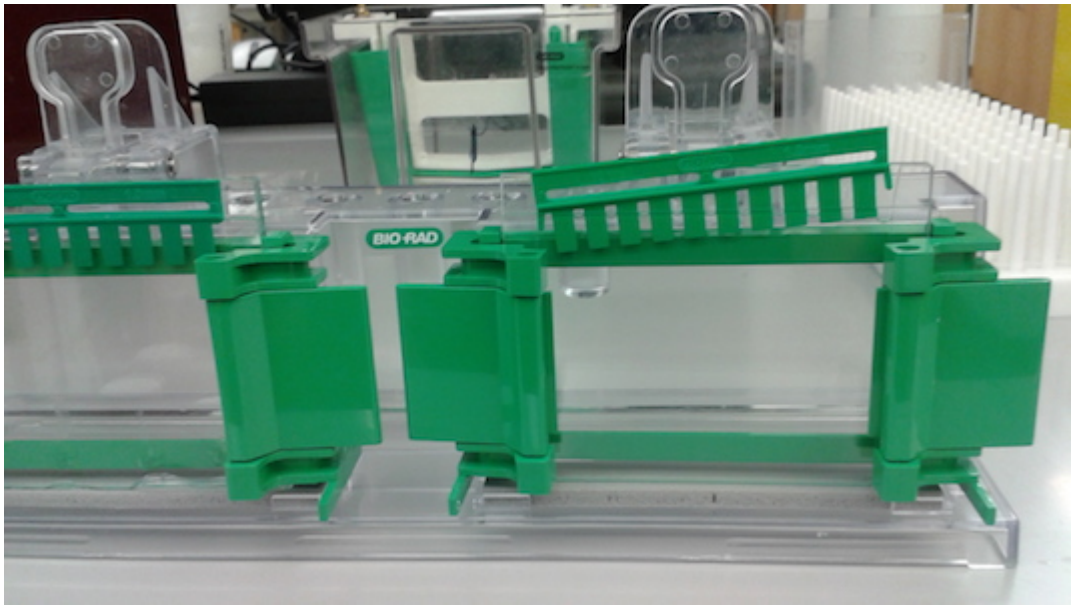
Handcast native polyacrylamide gels

Step 3.

To cast two minigels, add in order: 7.8 mL water, 1.2 mL 5X TBE, 3.0 mL 38:2 acrylamide:bis, and 45 µL 10% APS to a 15 mL screw cap centrifuge tube. Mix by vortexing briefly. Once everything is ready, add 15 µL TEMED and vortex briefly to mix before loading gel solution into each of the two casting

assemblies. A 6” Pasteur pipette works well for this purpose. Stop before filling (2 mm from top). Carefully insert 10-well comb, using a slight angle to avoid trapping any air, and seat completely. Free-radical polymerization of these gels should be complete in 15 min at room temperature, and these minigels should be ready for use immediately after that. Note: aforementioned 38:2 acrylamide:bis volume is for casting two 10% polyacrylamide gels. Should a different final acrylamide concentration be desired, adjust it and water volumes accordingly.





Handcast native polyacrylamide gels

Step 4.

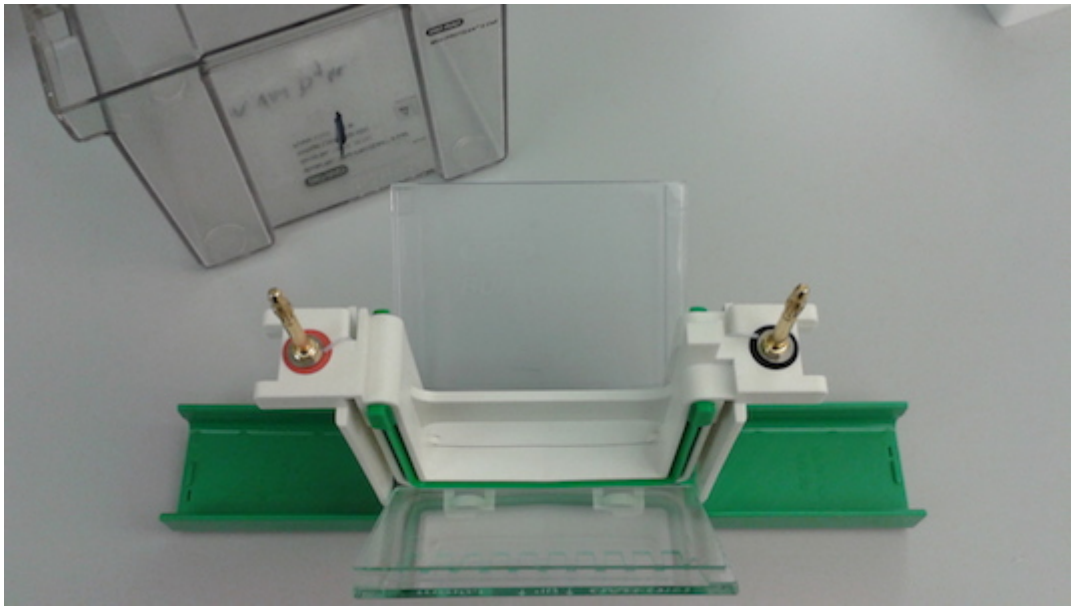
Note: polyacrylamide minigels in their casting stands can be kept at room temperature/humidity for several hours without any problem. Long-term storage is best when hydrated with a small quantity of water (200 µL on comb/small plate junction), wrapped in plastic and/or sealed in an airtight container,

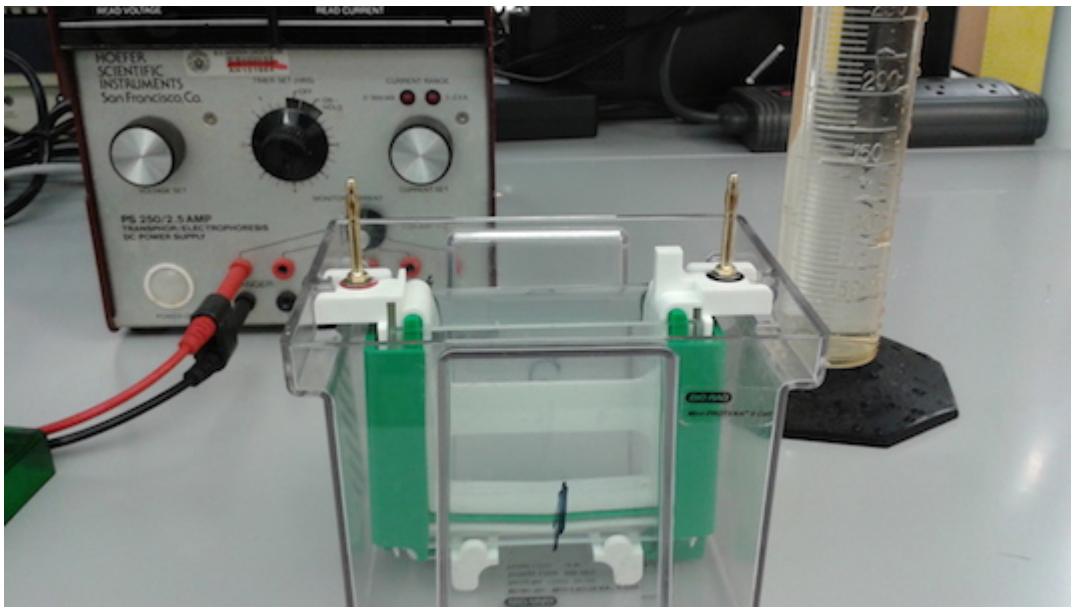
and stored at 4 ºC. Such minigels have effective lifetimes of at least 1 month if stored properly.

Polyacrylamide gel electrophoresis

Step 5.

With care, secure one polyacrylamide minigel and one buffer dam to each side of the Mini-PROTEAN® electrode assembly. Ensure the green gasket “ears” fully seal with the small plate/spacer junction of the minigel and corresponding plastic lip of the buffer dam before closing side clamps. Place assembly into the tank. Note: we routinely use smaller Mini-PROTEAN® II tanks for buffer economy; adjust subsequent volumes accordingly. Prepare 500 mL ½X TBE buffer. Fill the central chamber with ½X TBE buffer and observe if any leakage occurs. If so, drain and repeat seating minigel and buffer dam. Note: if two gels are to be electrophoresed simultaneously, substitute buffer dam for a second minigel.





Polyacrylamide gel electrophoresis

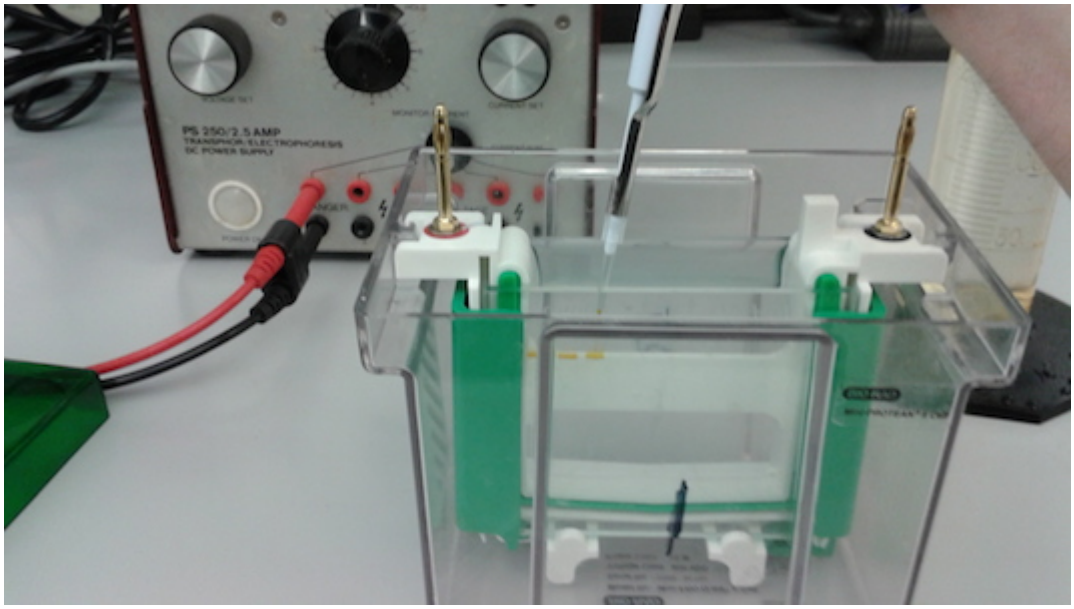
Step 6.

Prepare DNA samples for electrophoresis. Total volume should not exceed 12 µL and contain at least 3.3% glucose and 0.025% Orange G dye final. **Note**: do not use blue or cyan loading dyes, as many fluoresce in the infrared. Thus, for a 5 µL binding reaction, we add 1 µL 6X Orange Loading Dye (20% glucose, 0.15% Orange G, stored –20 ºC), and mix thoroughly by repeated pipetting.

Polyacrylamide gel electrophoresis

Step 7.

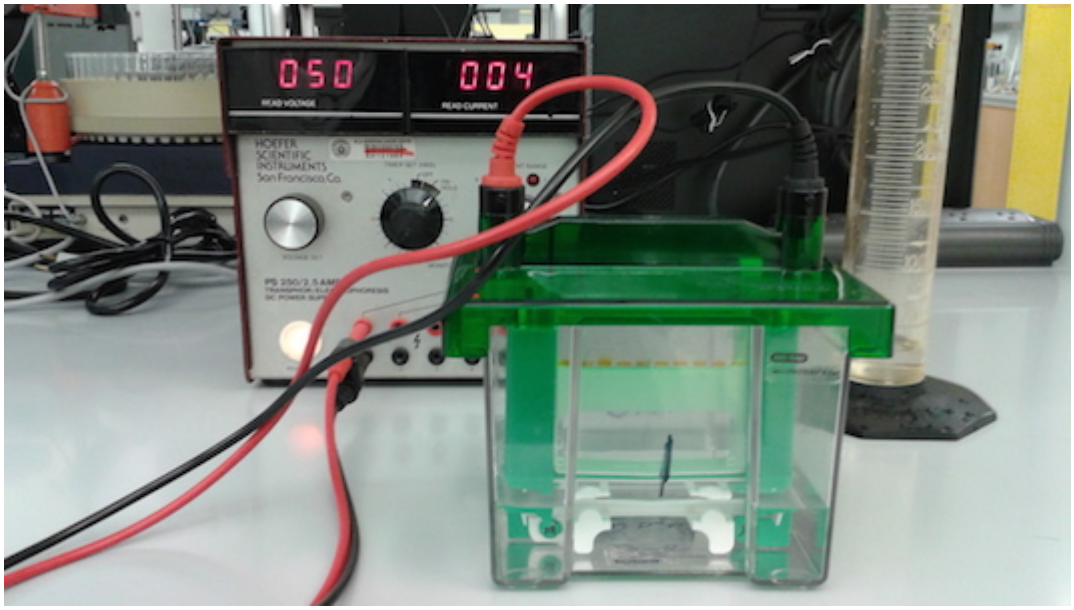
Samples are loaded in order into their respective wells using a P10 Pipetman and 0.1 - 10 µL standard tips. Once all samples are loaded, ½X TBE buffer is added to the bottom chamber, the tank lid seated, and leads attached to a power supply. **Note**: be aware of lid-to-electrode assembly and lead-to-power supply socket orientations to maintain proper electrode polarity.



Polyacrylamide gel electrophoresis

Step 8.

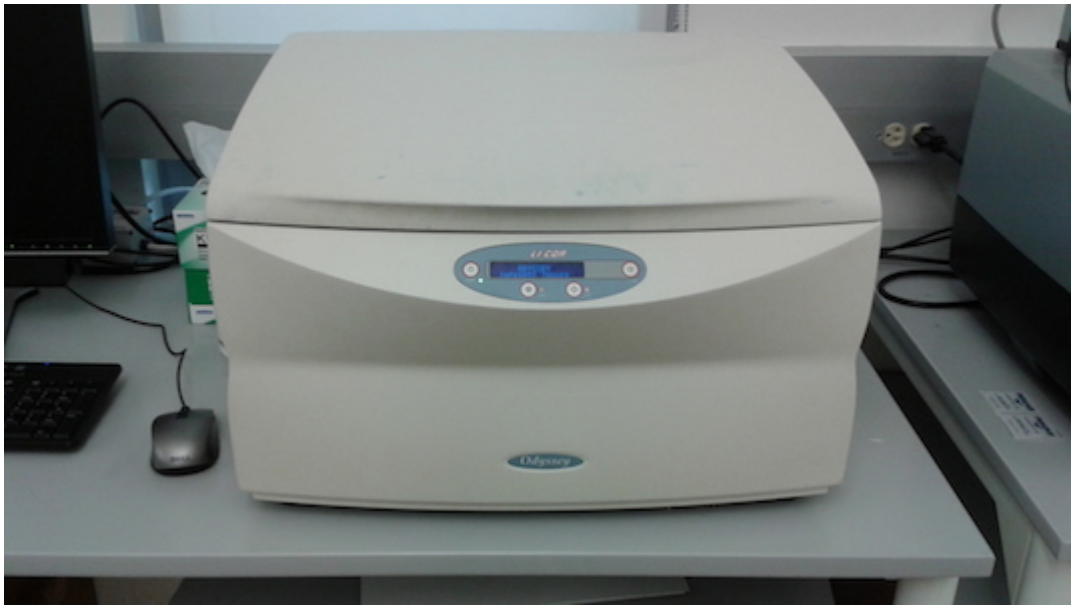
Electrophorese samples at constant voltage 5 V/cm (50 V) for 5 min to allow the DNA to enter gel, then increase the voltage to 10 V/cm (100 V) for the duration of the run. One hour is sufficient to deliver the Orange G dye to near the bottom of the minigel, which corresponds to approximately 10 bp dsDNA. Once the run is completed, drain tank and electrode assembly, wash with water and disassemble. **Do not take apart gel**, as they can be directly imaged while between glass plates. Note: use of a power supply with integrated timer is a helpful convenience, preventing sample overruns and facilitating reproducibility between runs. However, do not let gels remain at room temperature for extended periods of time, as diffusion of shorter DNAs can occur.

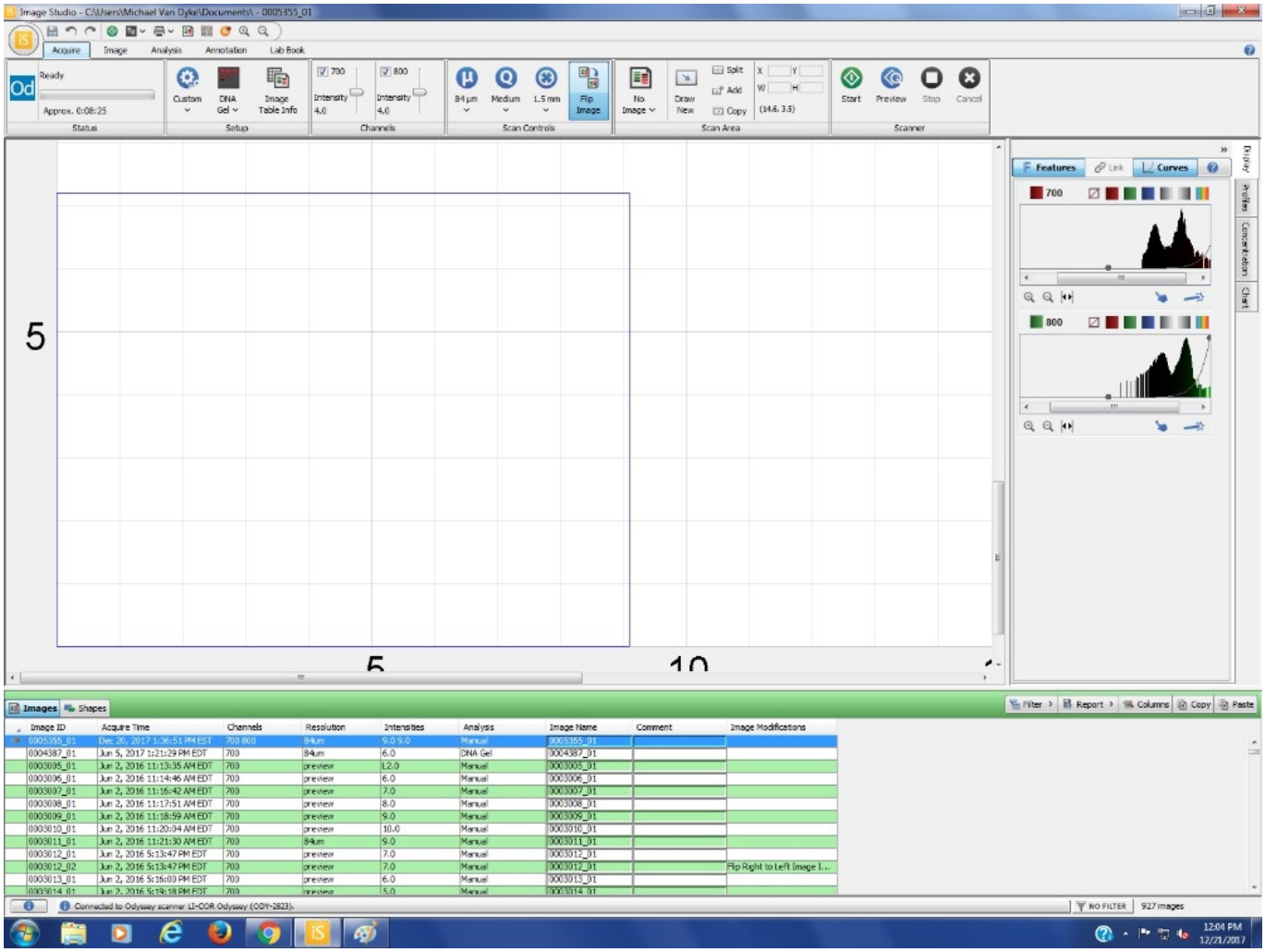


Infrared fluorescence imaging

Step 9.

To perform infrared fluorescence (IRF) imaging of PAGE minigels, we use a LI-COR Odyssey imager and LI-COR Image Studio™ 5.2 software. In Image Studio™, the DNA preset is used with Scan Controls settings: Scan Resolution (μ): 84 μm, Scan Quality (Q): Medium, Focus Offset (⊕): 1.5 mm, Flip Image. A 7 × 9 cm grid is selected corresponding to the minigel, typically the leftmost, bottom corner of the available field. Check the appropriate channels for the IRDye®-labeled DNAs present. Initial Intensity settings will depend on the quantity of labeled DNA present in each reaction. We use the general guideline: 10 fmoles (Intensity 9), 100 fmoles (Intensity 6), 1000 fmoles (Intensity 3).

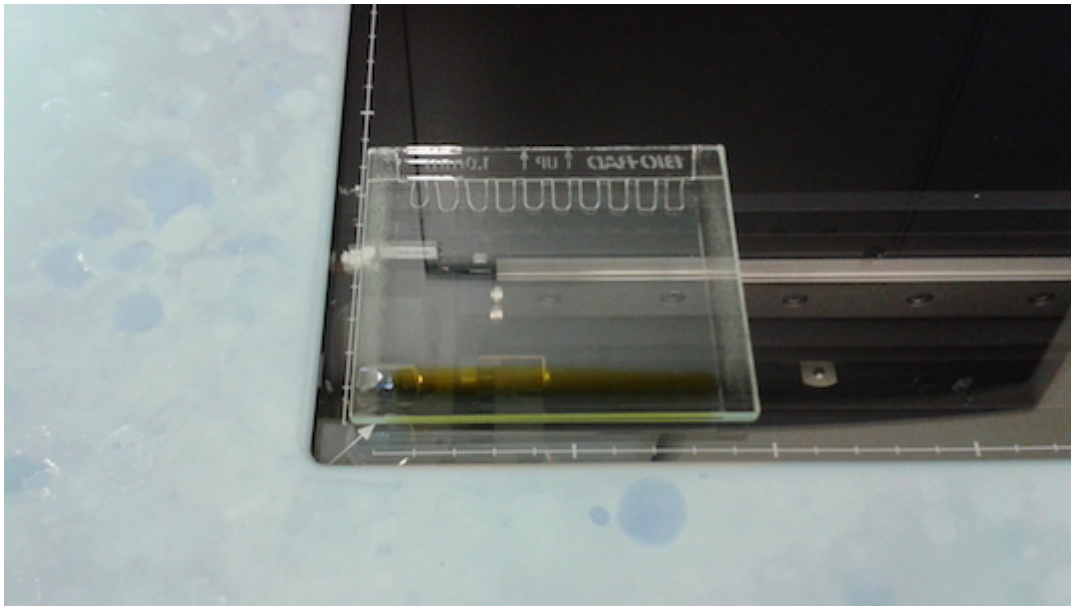




Infrared fluorescence imaging

Step 10.

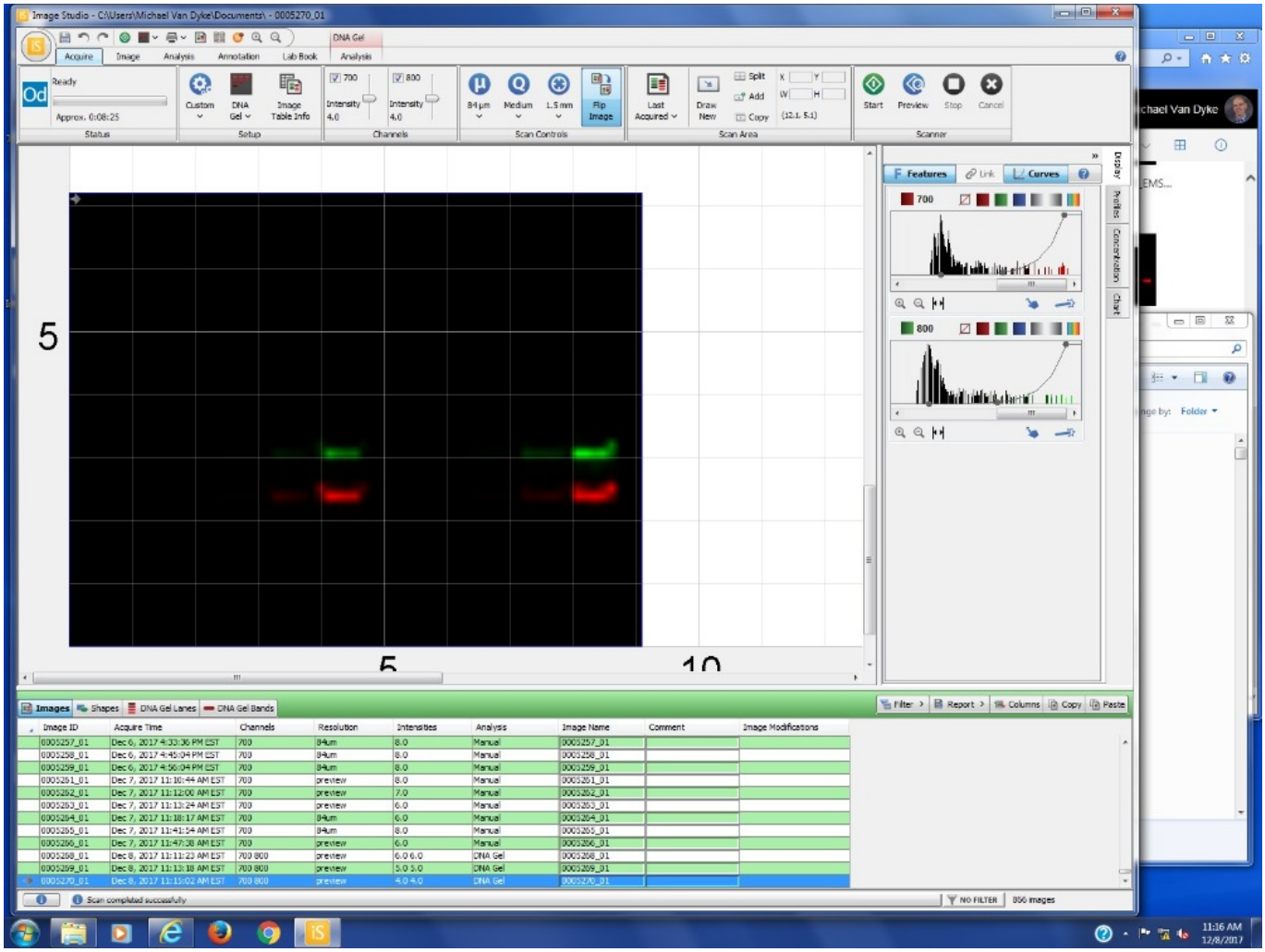
Note: before imaging, make sure the glass surface of the imager is completely clean. Use DI water and Kimwipes™ to ensure minimal background spotting when should the gel be imaged at high intensity or subjected to high gain enhancement. Place the clean gel sandwich onto the glass imager surface with the short plate face down. Align with both axis and ensure the gel location corresponds to that indicated in Image Studio™. Close imager lid.



Infrared fluorescence imaging

Step 11.

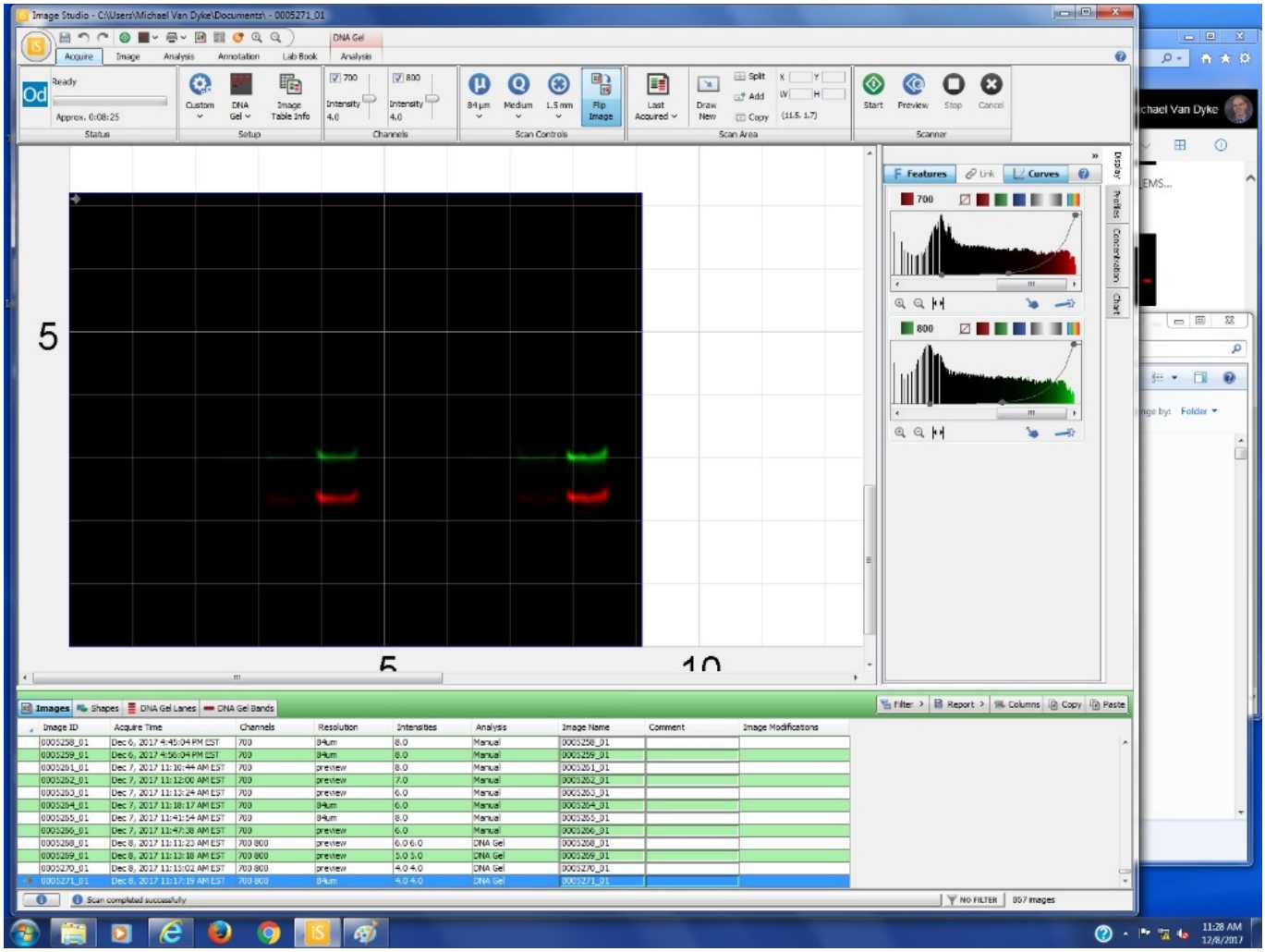
Initially, a Preview scan at Intensity 4 for both 700 and 800 channels (as needed) is performed, which takes approximately 30 s to run. Additional Preview runs at increasing or decreasing intensity are performed, as needed, until no saturated pixels (white) are observed. Once maximal Intensity values have been determined, a full run may be initiated (Start). Such takes approximately 8.5 min to run.

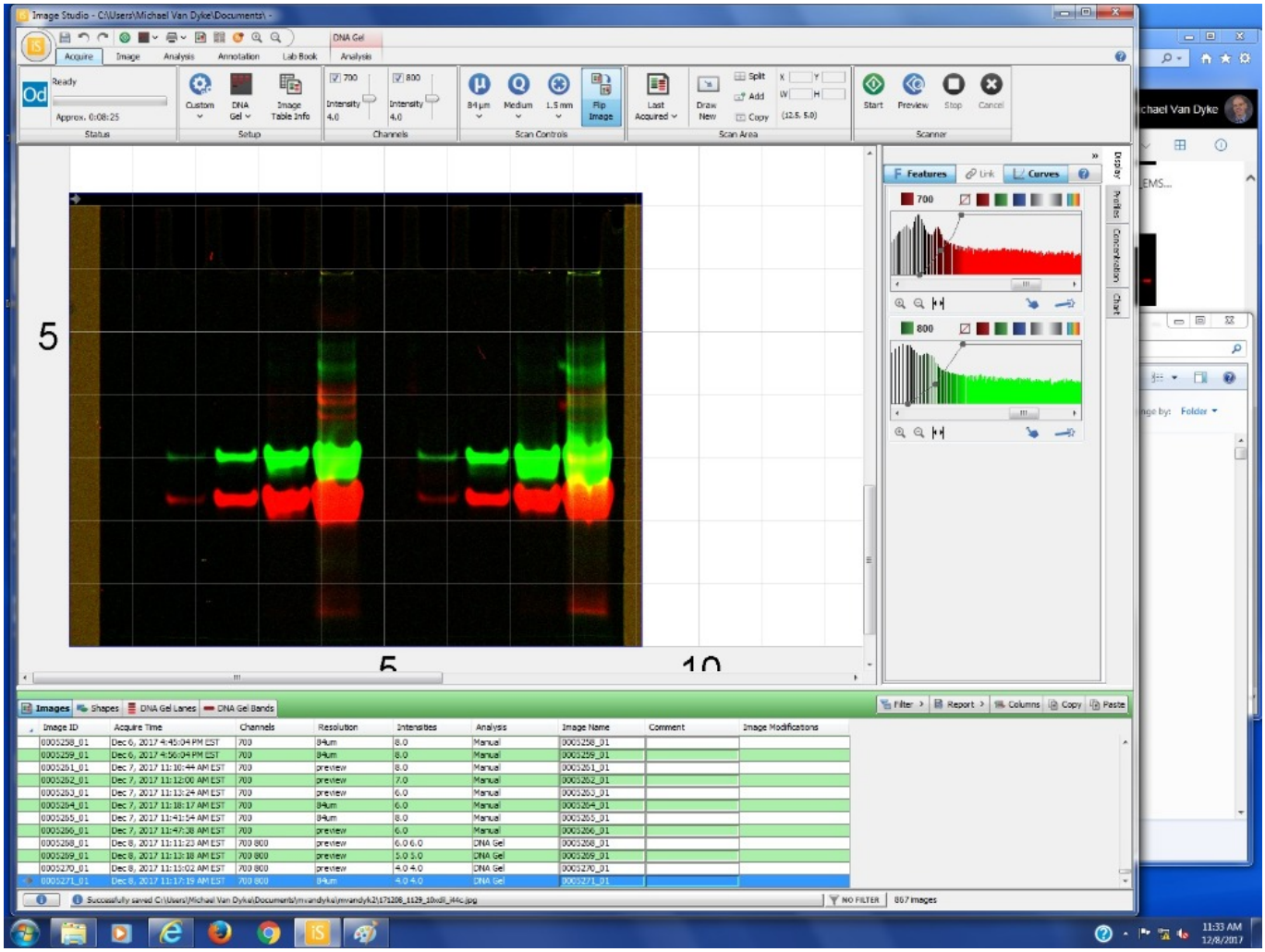


Infrared fluorescence imaging

Step 12.

Images can be saved as tif, png, jpg files and at 150, 300, 600 dpi resolution. Our standard is a 600 dpi jpg file. Gain can be adjusted for each channel using the Curves box, at right. While this allows observation of weaker bands, it is an image manipulation that may not be suitable for publication.

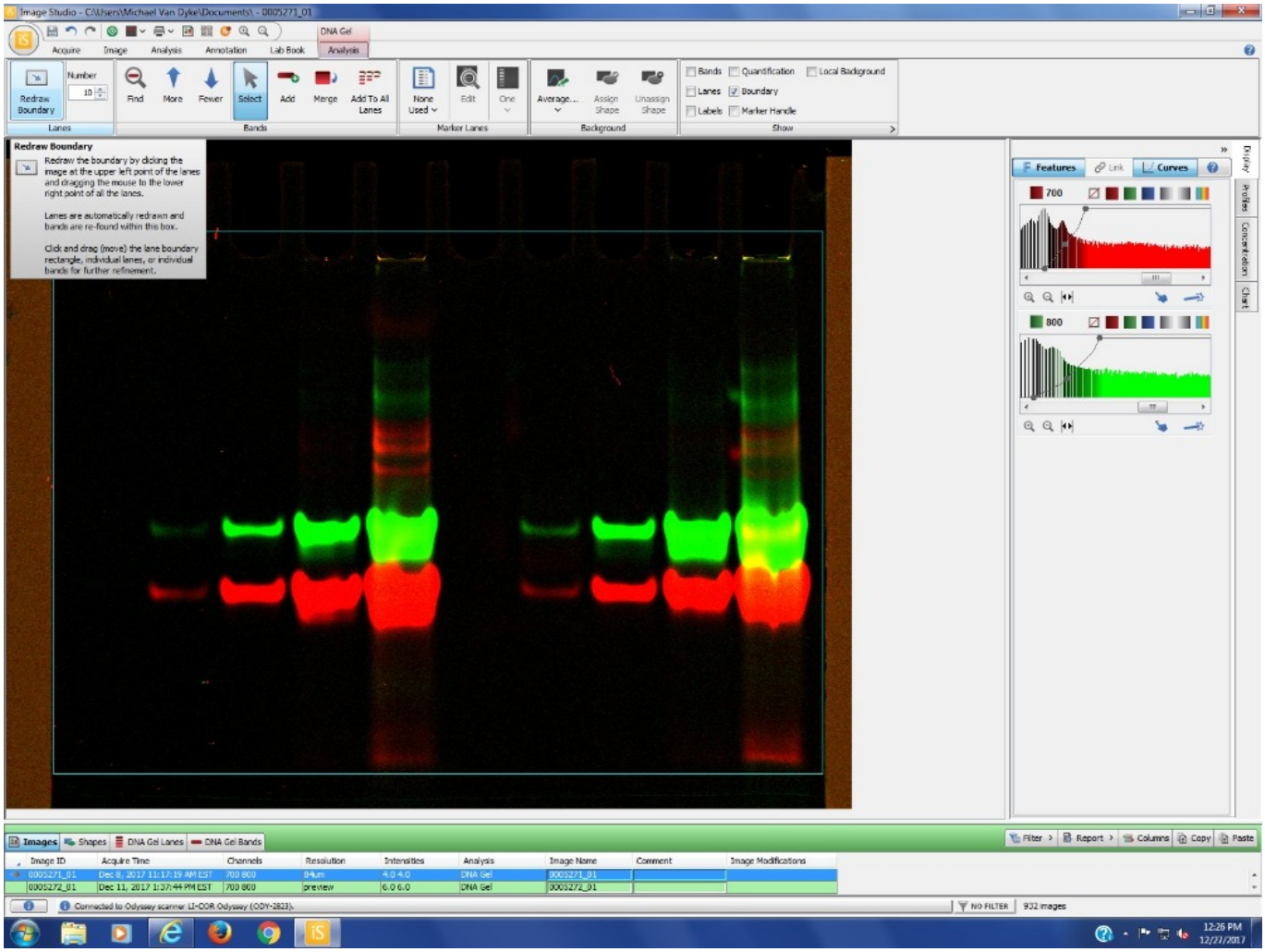


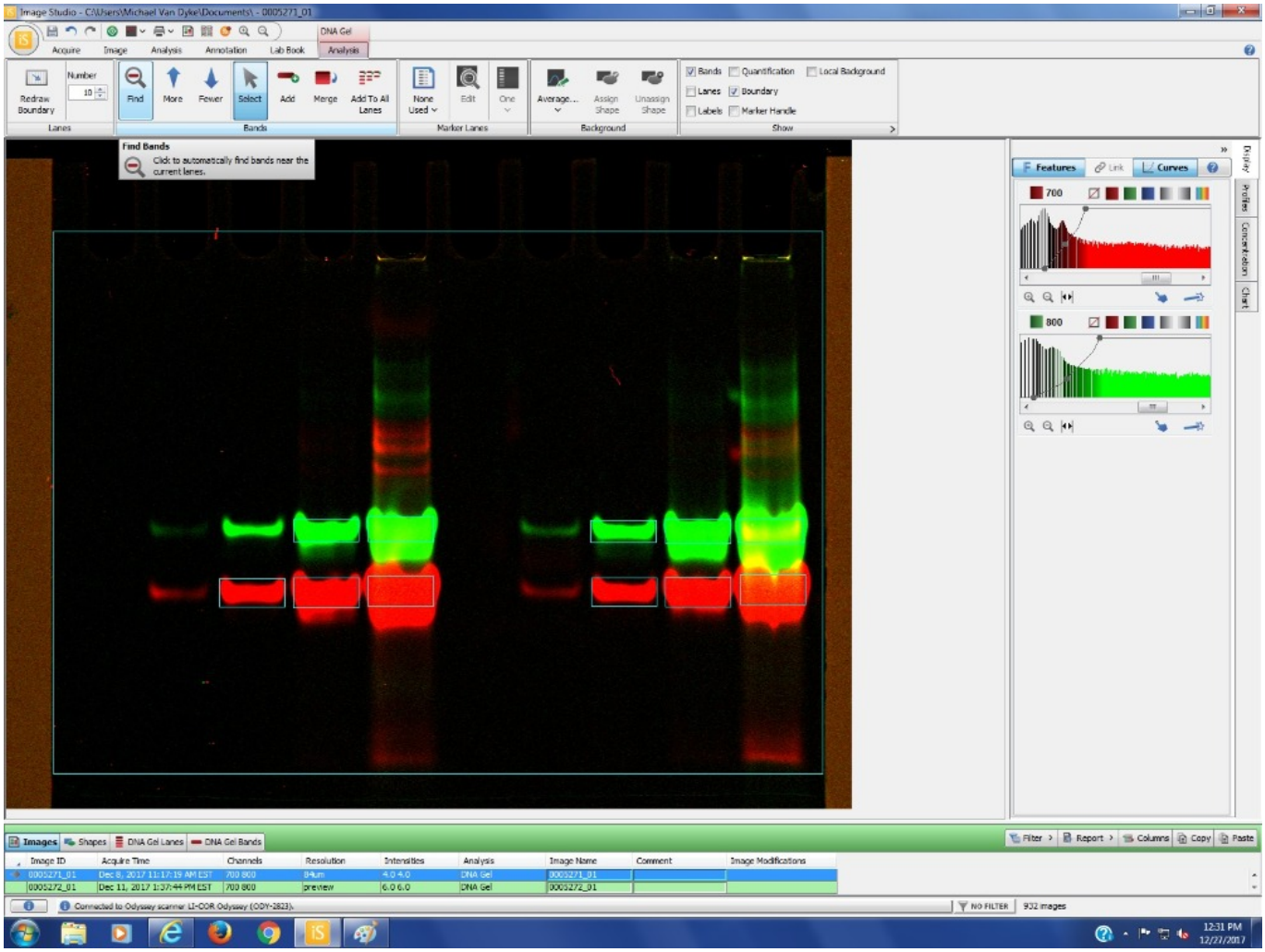


Infrared fluorescence quantitation

Step 13.

Image Studio™ software provides the capability to quantitate image bands. Note that gain adjustments do not affect these values, so long as no saturated (white) pixels are present. Bands may be automatically identified using the DNA Analysis tab or manually, using the Analysis tab. To use the DNA Gel:Analysis function: (1) Click on the appropriate tab. (2) Click on Lanes:Redraw Boundary to draw a rectangular boundary around the bands of interest. (3) Indicate the number of lanes present. Our default is 10. (4) Click on Bands:Find to find those bands you wish to quantitate. A gray, equivalent sized rectangle will be drawn around each chosen band. Click on Bands:More to identify additional bands for quantitation, as needed.

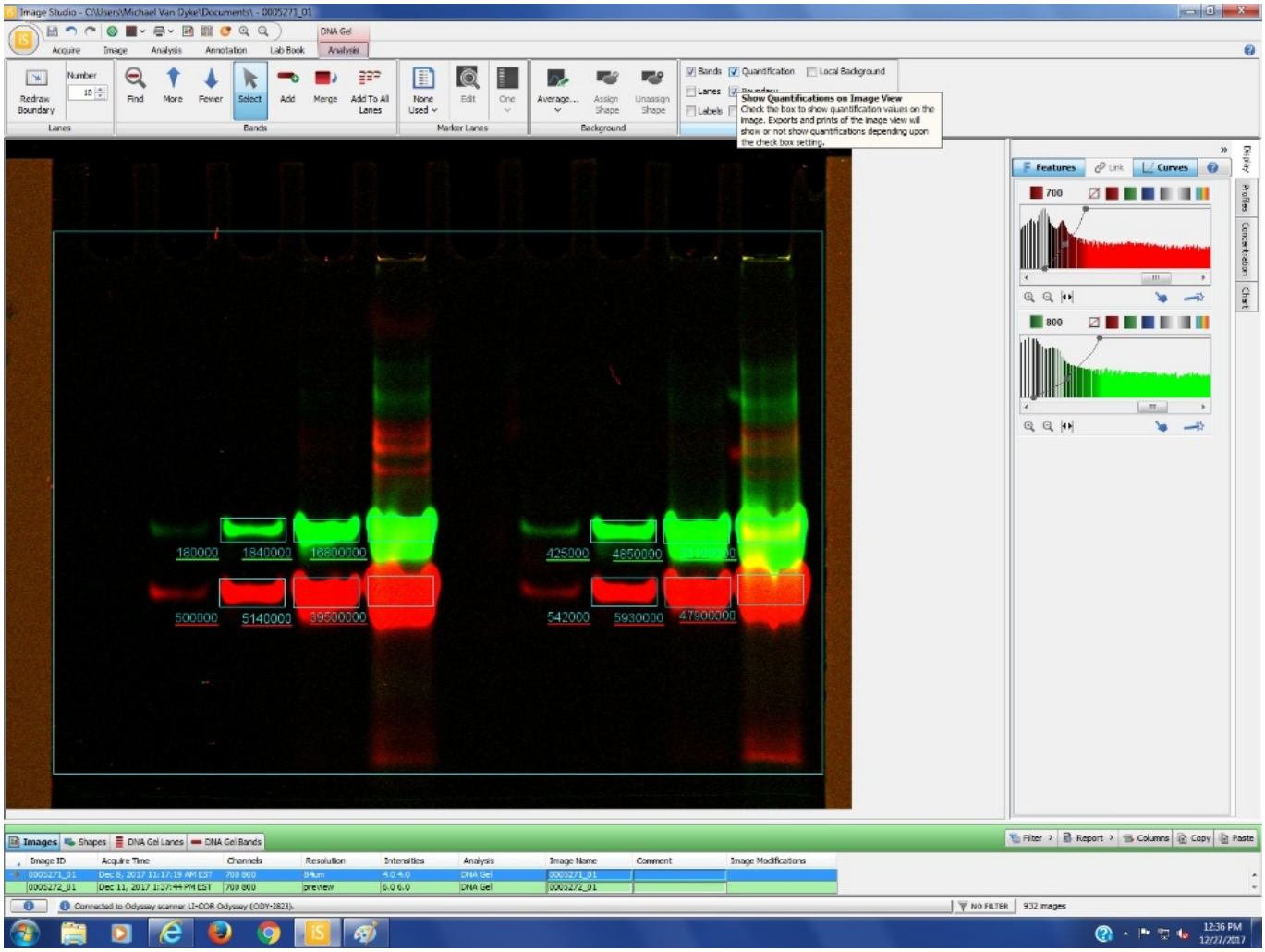




Infrared fluorescence quantitation

Step 14.

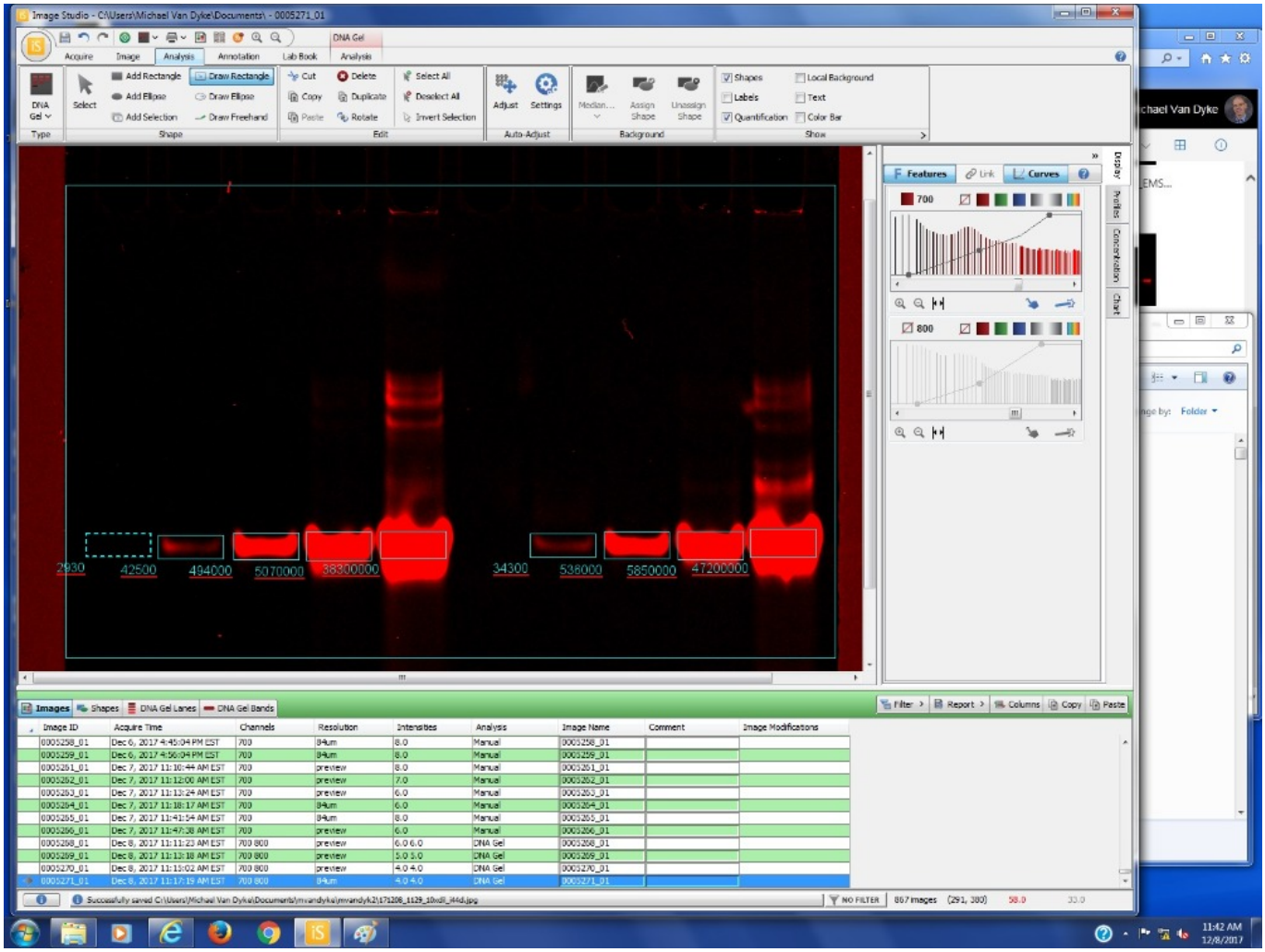
(5) Once the full complement of bands has been identified, click on Show:Quantification to obtain the pixel numbers for each quantitated band. Note the color bar under each value as these indicate the channel (700 or 800) being quantitated. (6) Remember to save a final image including quantitation values for your records. Occasionally, channel gain (Curves) may need to be reduced to obtain clear images of the pixel numbers. Note that pixel numbers do not change with changes in channel gain.

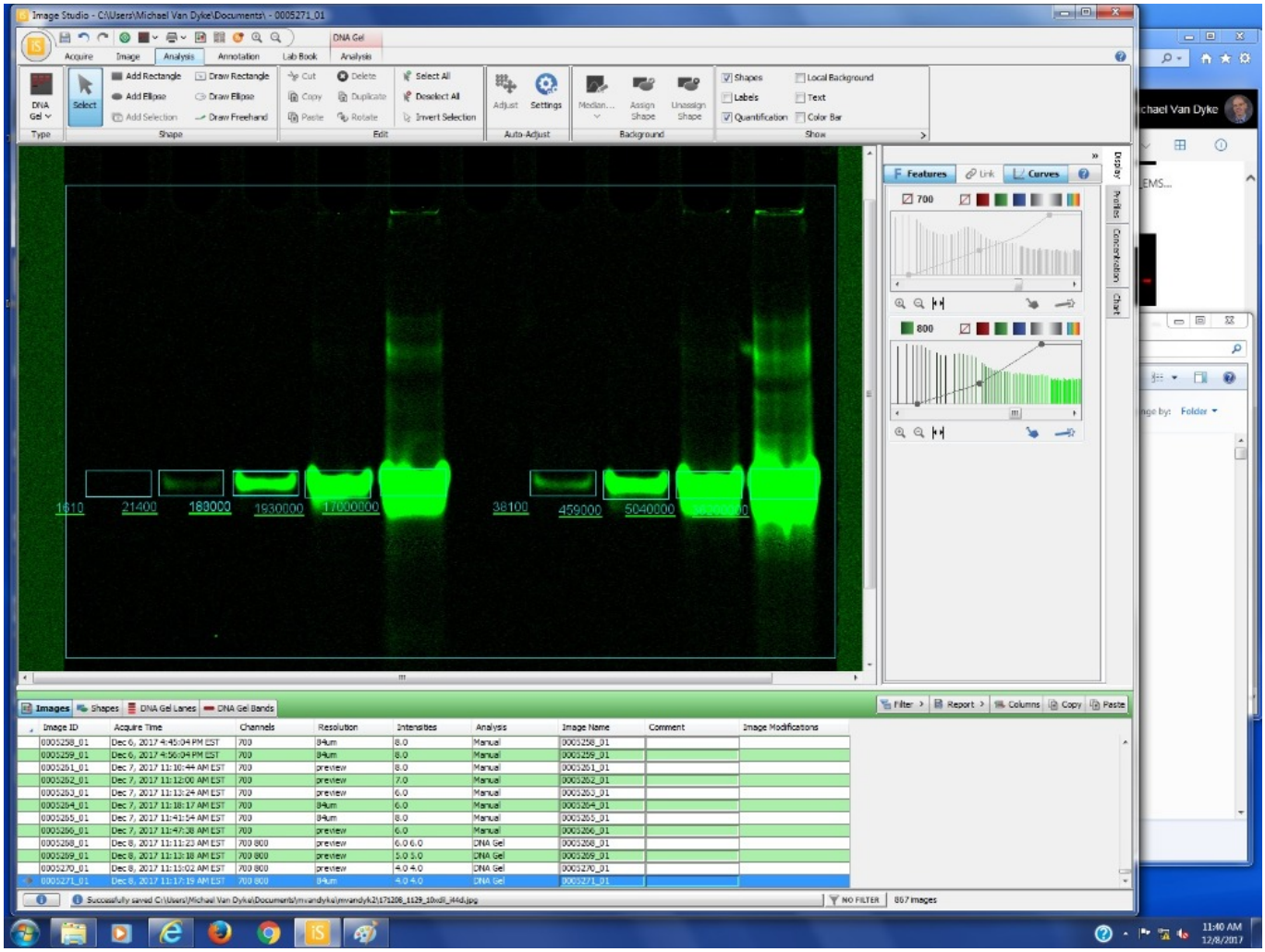


Infrared fluorescence quantitation

Step 15.

Under some circumstances (e.g., quantitation of faint bands or background), it is necessary to perform band quantitation manually. Such may be accomplished clicking on the Analysis tab. Use the Shape:Draw Rectangle function to enclose additional bands of interest or background. Take care to ensure all rectangles are of equal dimensions. Note: Analysis requires identifying bands one channel at a time, although both channel analyses may be shown simultaneously once completed. Remember after quantitation to save final images, thereby providing a record of pixel numbers for each band.





Warnings

Acrylamide dust is an inhalation hazard and cumulative neurotoxin. Best to wear a protective face mask when weighing.