



# **Refraction-2D™**

## **Detailed Spot Picking Guide**

For manual picking of protein spots from 2D gels

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## 1 General information

The Refraction-2D™ Spot Picking Kit allows the accurate and uncomplicated picking of fluorescence labeled protein spots without further gel staining.

Spot picking can be performed with a fluorescence-supported spot picking robot as well as manually. The spot picking can be done from self-casted gels as well as from pre-casted gels. Gels can be foil supported, or placed on foils or glass plates.

Since the principle of minimal labeling is based on the tagging of only 3% of proteins, the co-migration of labeled proteins with conventional labeling dyes (like cyanines) is rather insufficient. For the picking of protein spots that are not labeled with G-Dye 100 an additional staining is crucially required for spot picking from proteins <40 kDa.

Due to the extremely low molecular weight of G-Dye 100 (0.3 kDa), a SDS-PAGE co-migration of G-Dye 100 labeled protein and the unlabeled counterpart can be observed for proteins with a molecular weight of approx. 5 kDa\*. Use at least 100 µg of G-Dye 100 labeled protein sample for spot picking. To improve sequence coverage of your protein identification by mass spectrometry, we recommend to add unlabeled sample to your regular Refraction-2D™ analysis or to prepare a preparative 2D gel (see Detailed Guide 4.6).

\*This applies only for polyacrylamide gels until a concentration of 12.5 %



### **For users of fluorescence supported spot picking devices:**

Use the fluorescence signal from G-Dye 100 for spot picking.  
The maximum excitation is at 498 nm, and the maximum emission is at 524 nm.

### **For users isolating protein spots manually:**

The manual Refraction-2D™ Spot Picking-Technology is based on a 1:1 printout of your 2D gel image with G-Dye 100 labeled protein spots. This printout serves – placed under your non-stained 2D-Gel – as exact picking template. For further information please refer to the present Refraction-2D™ Spot Picking Guide.

The Refraction-2D™ Spot Picking Kit and Refraction-2D™ Labeling Kit contain an (extra) vial G-Dye 100 SP (for spot picking) sufficient for labeling of 150 µg of protein.

### **To prepare G-Dye 100 SP working solution:**

1. Allow vial containing G-Dye 100 SP to warm up to room temperature.
2. Spin down vial briefly.
3. Dissolve G-Dye 100 SP in G-Dye solvent (3 µl per vial to get a final concentration of 400 pmol/µl).
4. Vortex and spin down briefly. G-Dye working solution is now ready for use.
5. For labeling instructions please read the Refraction-2D™ Product Guide\* (Use 1 µl G-Dye 100 SP working solution for labeling of 50 µg protein).

\*download:[http://www.dyeagnostics.com/site/wp-content/uploads/2011/01/Refraction-2D™\\_Product-Guide.pdf](http://www.dyeagnostics.com/site/wp-content/uploads/2011/01/Refraction-2D™_Product-Guide.pdf)

## 2 Detailed Guide

### 2.1 What you need

- G-Dye 100 camera or scanner images in exportable tiff format
- Image processing software enabled to change the resolution of tiff images (Adobe Photoshop or the freeware Irfan View)
- Ink jet- or laser printer (min. DIN A4)
- Low fluorescence glass plate or low fluorescence gel cassette (Prod. no. PR 04)
- Spot picker
- Fixating solution (40 % ethanol, 10 % acetic acid)
- Storage solution (25 % ethanol, 3 % glycerol)
- Picking solution (25 % ethanol)
- When using pre-casted gels, please refer to the instructions of the respective supplier and as well the chapter Tips & Tricks (see. 4.4)

### 2.2 Precautions

Use gloves and a hairnet in order to avoid keratin contaminations when working with your gel.

Avoid delays between scan (II) and the spot picking process since distortions of the gel can occur and disturb the matching of the picking template and the gel.

We recommend to practice spot picking with the provided spot picking example (see chapter 3) upfront, in order to receive satisfying results, and to get used to the technology described by the Refraction-2D™ Spot Picking Guide.

## **2.3 Detailed spot picking overview**

### **2.3.1 2D gel electrophoresis**

Perform your Refraction-2D™ analyses as usual. For subsequent protein identification per MS we recommend to scale up the protein quantity by the addition of unlabeled protein sample prior to IEF or the preparation of preparative gels with G-Dye 100 labeled proteins 100 (Note, the Refraction-2D™ Labeling Kit (≥12G) provides an extra aliquot of G-Dye 100 for spot picking; see also Tips & Tricks). Maximum recommended protein amount for large 2D gels (24 cm IEF strip): 1 mg (Please refer as well to the supplier specifications for your IEF strips).

### **2.3.2 Scan (I) and fixation/ storage of the gel**

Conduct the scanning or imaging of your 2D gels as usual. Fix the gel for 30 min with fixating solution (40 % ethanol, 10 % acetic acid) and store the gel subsequently with storage solution (25 % ethanol, 3 % glycerol) protected from light.

### **2.3.3 Evaluation and spot selection**

Conduct the evaluation and spot selection as usual with your 2D software

### **2.3.4 Preparation and marking of the gel**

In order to match your gel and the 1:1 size matched print-out you have to mark the gel. Rinse the gel first for 15 min with Picking solution (25 % ethanol) and place it subsequently onto a low fluorescence glass plate. Humidify the gel with Picking Solution in order to avoid drying and distortion. If your gel is kept humid on the glass plate, it will retain its original shape for at least 1 hr. Use your spot picker to mark the gel with one hole in three corners of the gel. The holes can be easily found in your scanned gel image (see as well: Quick Guide 2.2 Fig. 1).

### 2.3.5 Scan (II) and storage of the gel

Scan the whole gel (including the low fluorescence glass plate) with the channel for G-Dye 100. Make sure to write down the scanning resolution (We recommend: 100  $\mu\text{m}$  per pixel; Fig. 1). Export the image file without format changes as tiff file. Cover the gel until spot picking if possible, in order to avoid contaminations.

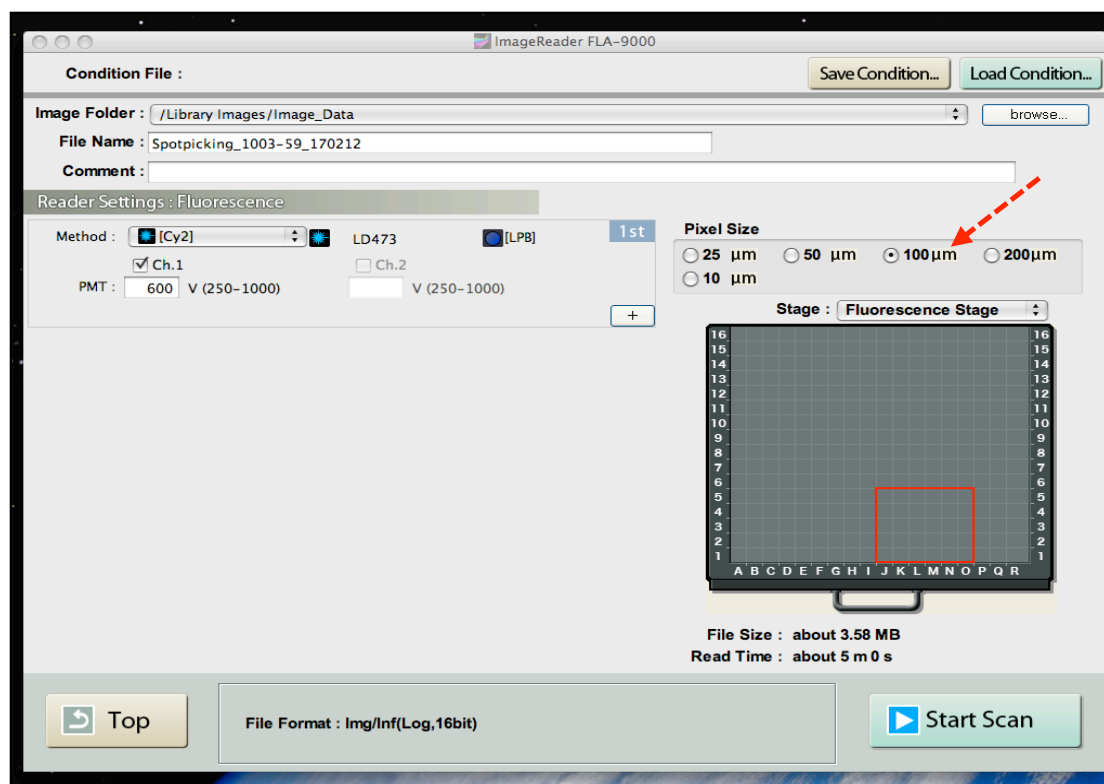


Fig. 1: Scanning parameters: Fujifilm FLA-9000 Image Reader (the red arrow points to the scanning resolution; screen shot)

### 2.3.6 Creating the picking template

To create a picking template you produce a 1:1 size-matched print-out copy of your scanned gel. For this, the print resolution and the scanning resolution have to be the same. Adapt the resolution of the tiff image with your imaging software (e.g. Adobe® Photoshop®). If you do not have an imaging software, we recommend to install and use the freeware Irfan View\*.

Proceed as follows:

- Open the respective image file
- Adapt the image resolution to the scanning resolution (please make sure that the image size is not recalculated by this, since the dimensions of the image will not match correctly).
- Print picking template (verify that the scale for printing is set to 100%).

Example:

Scanning resolution = 100 µm/pixel  
 = 10 pixel/mm  
 = 100 pixel/cm  
 = 254 pixel/Inch (dpi)

Scanning resolution = Printing resolution = 254 dpi

If scanned with 100 µm per pixel, set the resolution of the tiff image to 254 dpi in order to print out with the original gel size.

\* [http://www.chip.de/downloads/IrfanView\\_12998187.html](http://www.chip.de/downloads/IrfanView_12998187.html)

### 2.3.6.1 Use of Adobe® Photoshop®

1. Open the respective image file
2. Adapt the image resolution/image size

Choose in the menu “Image” the option “Image size”. The dialogue box “Image size” opens up. Change now in the field “Document Size” the “Resolution” corresponding to your original scanning parameters for scan (II) (100  $\mu\text{m}$  scanning resolution = 254 dpi respectively Pixel/inch; Fig. 2). Deactivate the option “Resample Image” (the corresponding dimensions in cm would not be adapted accordingly).

3. Print your picking template. Make sure to set the scale for printing to 100% (Fig. 3).

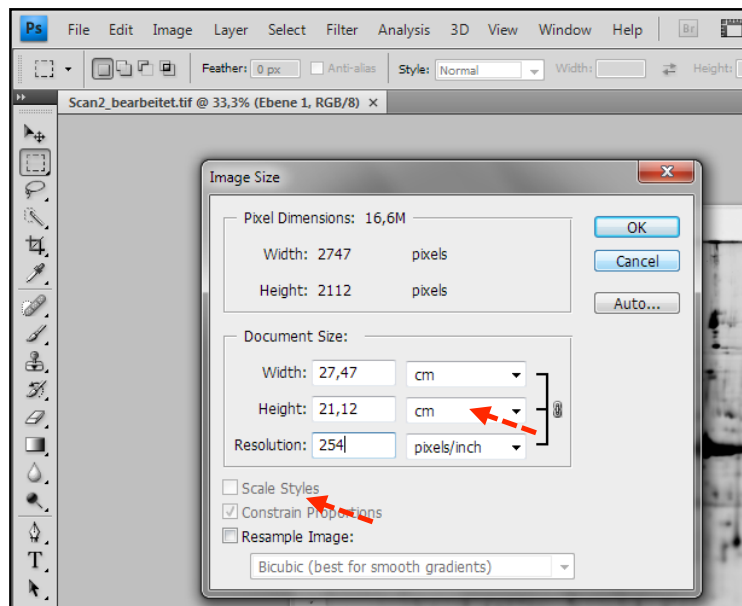


Fig. 2: Adapting the image resolution with Adobe® Photoshop® (Screen shot).

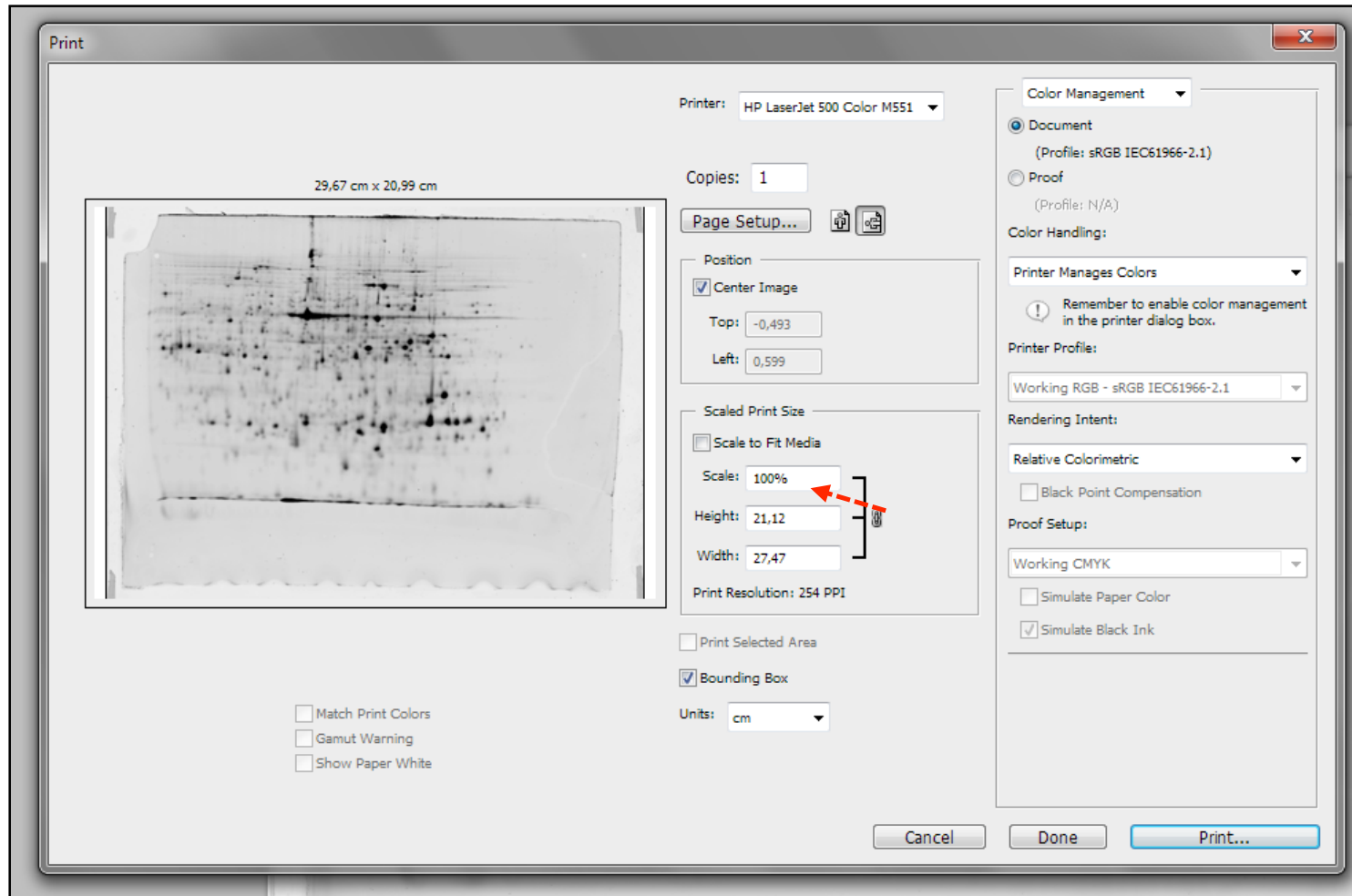


Fig. 3: Printing settings (screen shot)

### 2.3.6.2 Use of IrfanView

IrfanView is a freeware enabled to change the size of tiff images.

[http://www.chip.de/downloads/IrfanView\\_12998187.html](http://www.chip.de/downloads/IrfanView_12998187.html)

To create your picking template you proceed as follows:

1. *Download and install the software.*
2. *Open the respective image file.*
3. *Adapt the image resolution/image size.*

Chose in the menu “Image” die Option “Resize/Resample” (Fig. 4). The dialogue box “Resize/Resample” opens up. The present image size in cm is displayed (Fig. 5). Activate in the dialogue field “Set new size” the setting “Pixels” (Fig. 6). Change now the resolution “DPI” corresponding to your original scanning parameters from scan (II) (100  $\mu\text{m}$  scanning resolution = 254 dpi; Fig. 7). The number of pixels in the dialogue field “Set new size” must stay unchanged. If you activate now the setting “cm”, the image dimensions in cm should have been changed (Fig. 8). Click “OK” to accept the changes.

4. *Print your picking template*

Please note (Fig. 9):

- Portrait or landscape format
- Activate under “Printing size” the button “Original size”.
- Activate under “Position” the button “centered”.



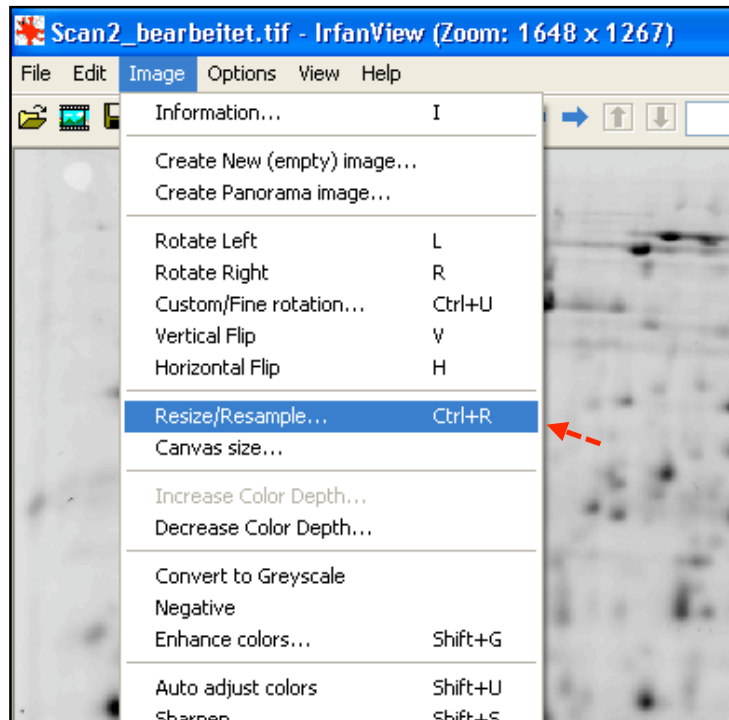


Fig. 4: Option „Change size“ in the menu „Image“ (Screen shot)

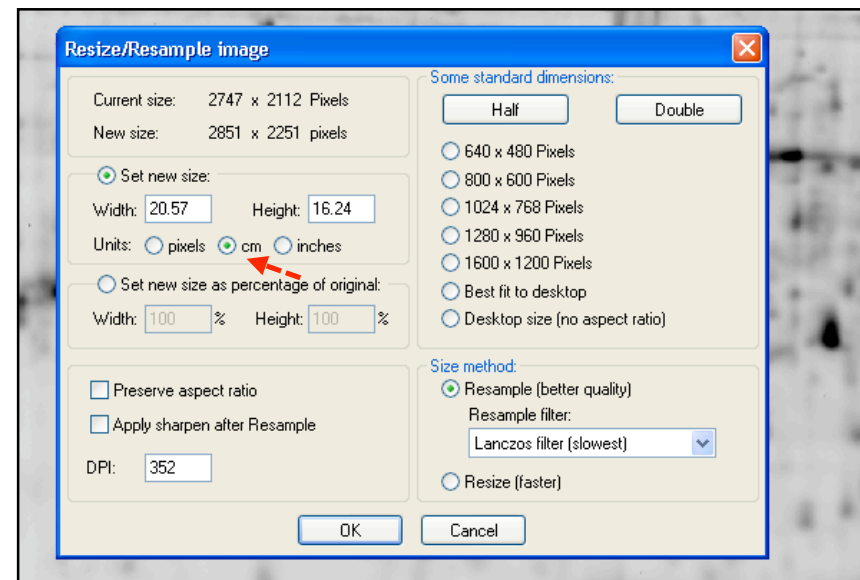


Fig. 5: IrfanView – Menu „Resize/resample“ (Screen shot)

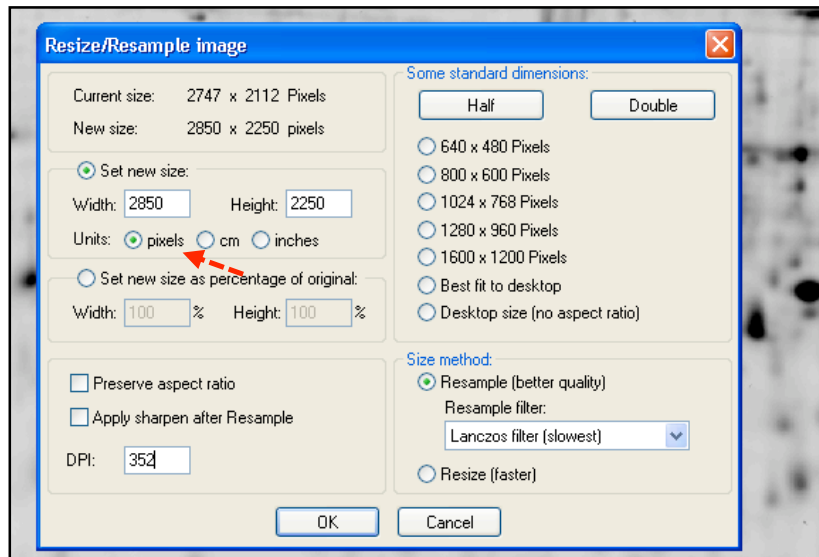


Fig. 6: IrfanView – menu „Resize/resample“ „Pixels“ activated (Screen shot)

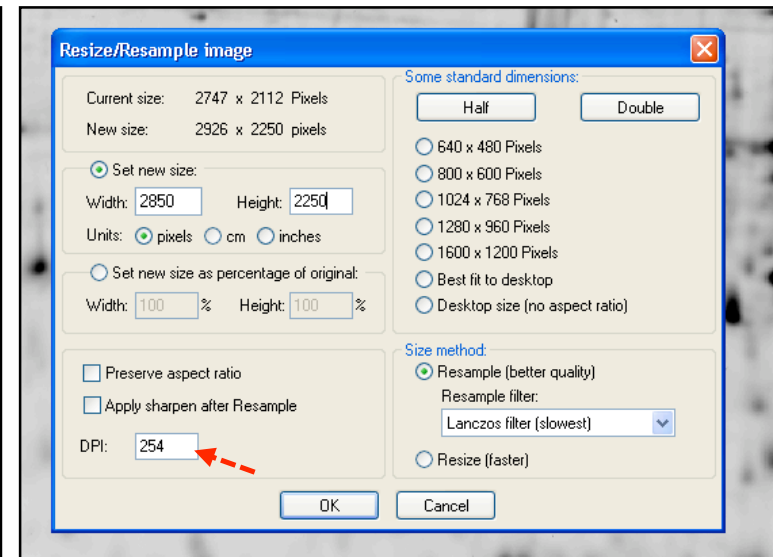


Fig. 7: IrfanView – menu „Resize/resample“, DPI adaption (Screen shot)

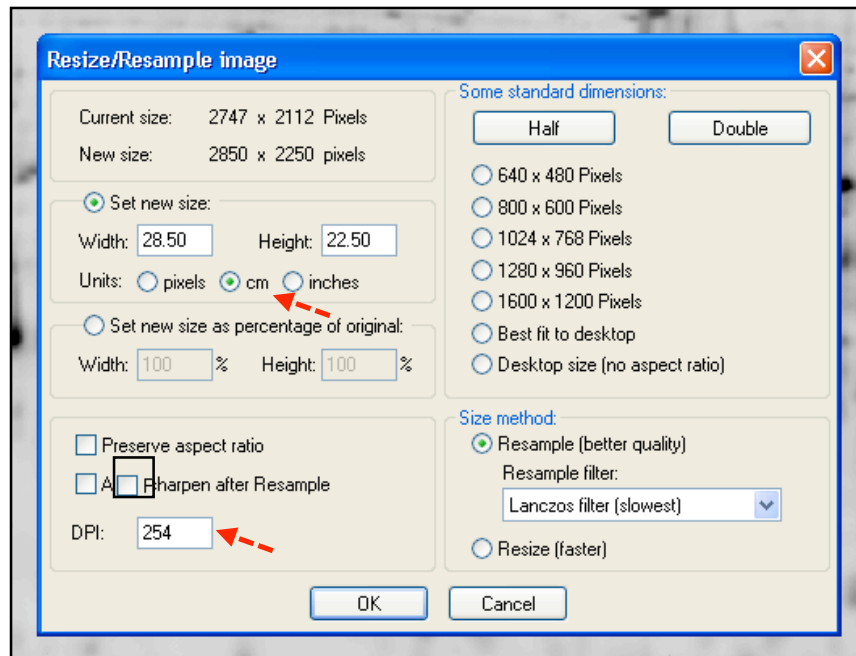


Fig. 8: IrfanView – menu „Resize/resample“, changed dimensions in cm (Screen shot)

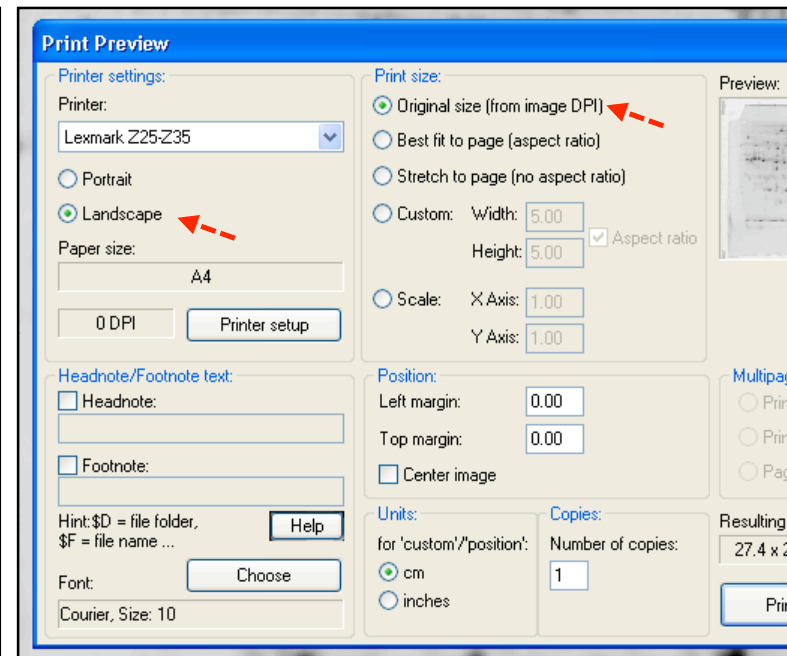


Fig. 9: IrfanView – menu „print“ (Screen shot)

### 2.3.6.3 Use of other Image Processing Software

Please make sure:

- (a) That the image size is not changed during the adaption of the image resolution, since the dimension of the image will not be adapted correctly.
- (b) That you set the scale for printing to „100 %“ or „not scale“ and not to „fit to paper size“.

### 2.3.7 Marking the picking template (optional)

We recommend to mark the spots of interest on your picking template in order to facilitate their selection during picking (Fig. 10). Furthermore, we recommend to encircle the marks in **the** corner of the gels in order to facilitate the exact matching of gel and print-out

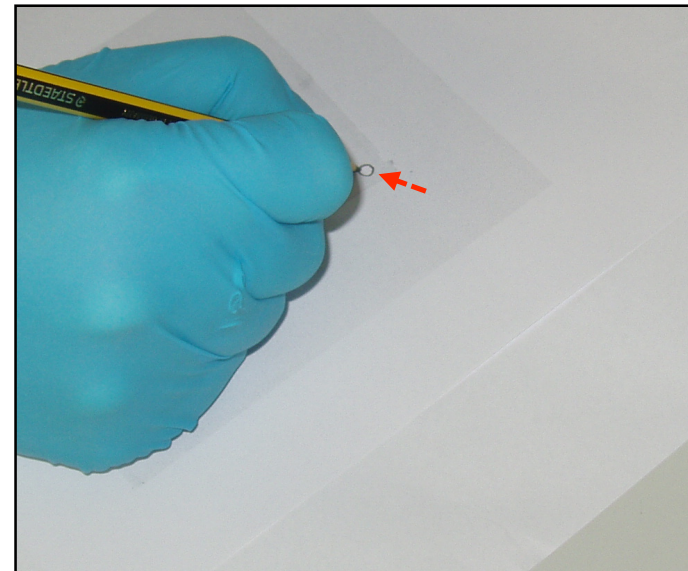


Fig. 10: Encircling of the marks in the corners of the gel on the picking template.

### 2.3.8 Spot picking

Place your spot picking template under the low fluorescence glass plate with your gel on it. Match the marks in the corners of your gel exactly with the encircled marks of the underlying printout (Fig. 11). Pick now your spots of interest. When you are finished, perform a control scan in the G-Dye 100 channel.

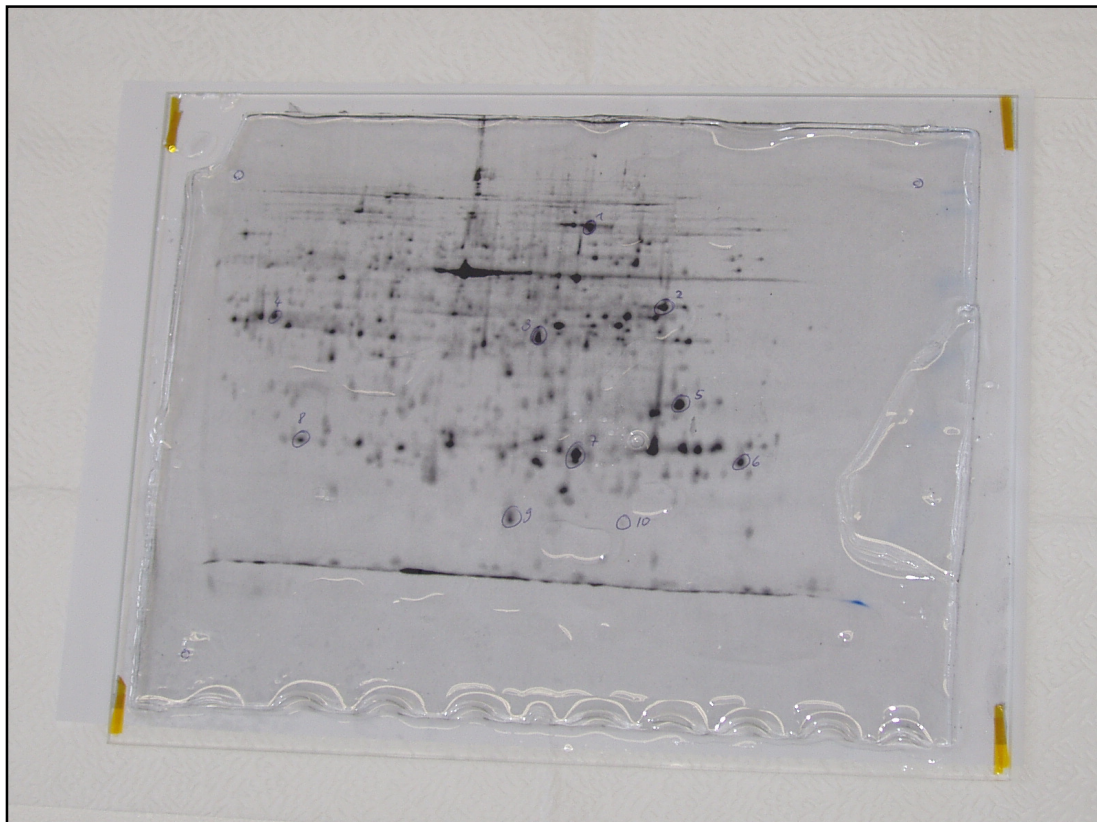


Fig. 11: Preparation for spot picking. The gel is placed on the picking template in order to perfectly match the marks in the gel and on the picking template.

### **3 Example for practicing: model protein BSA**

#### **3.1 2D gel electrophoresis**

Separate 2 µg of G-Dye 100 labeled BSA (see: Refraction-2D™ Product Guide) with a small 2D gel as usual (e.g. IEF: pH 3-10; SDS-PAGE: 13 % polyacrylamide gel). The Refraction-2D™ Spot Picking Kit contains an extra aliquot of G-Dye 100 for conducting a practicing example and for one preparative gel.

#### **3.2 Scan (I) and storage/ fixating the gel**

Scan the complete gel in the G-Dye 100 channel. This scan serves as a control of the quality of the 2D gel. Fix the gel for 30 min with Fixating Solution (40 % ethanol, 10 % acetic acid).

*If you wish to pick spots directly:*

Rinse the gel for at least 30 min with Picking Solution (25 % ethanol) and continue by marking the gel (see. 3.3; a 15 min rinsing is not required).

*If you wish to pick spots later:*

Store the gel protected from light in Storage Solution (25 % ethanol, 3 % glycerol) and continue with section 3.3.

#### **3.3 Preparing and marking of the gel**

Rinse the gel for 15 min with Picking Solution (25 % ethanol) and place it subsequently on a low fluorescence glass plate. Use your spot picker for marking the gel with one hole respectively in three corners. The holes can be easily detected later on in the scanned image (Fig. 12).

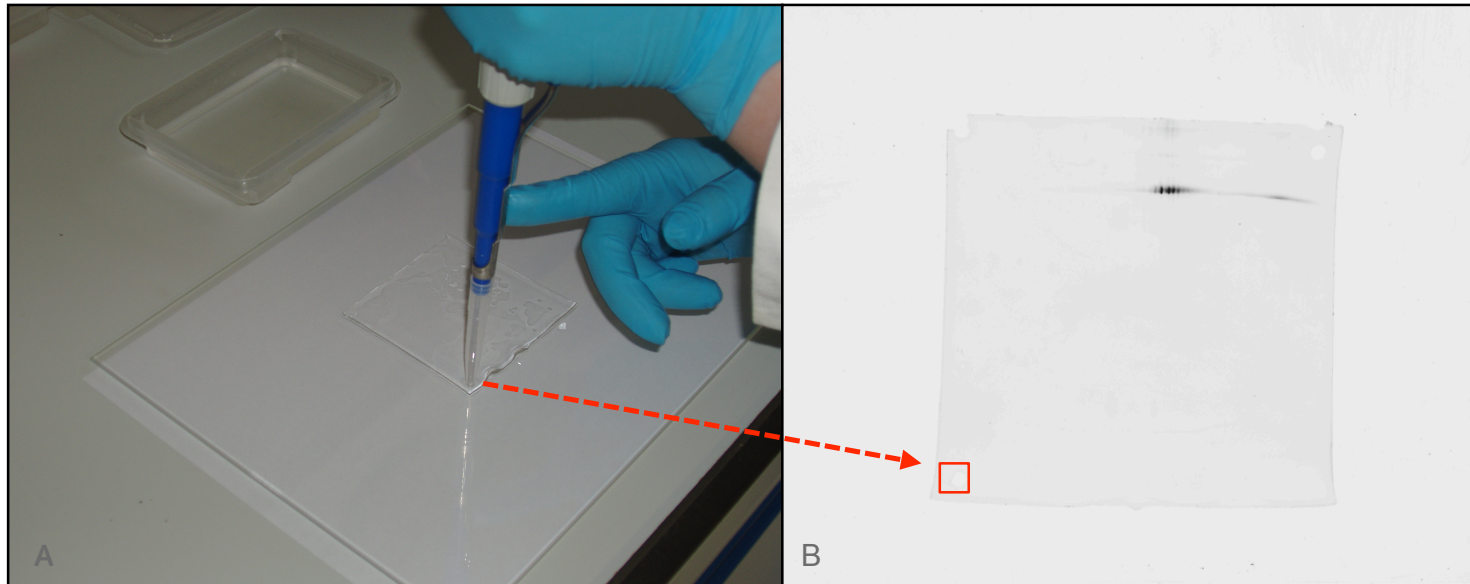


Fig. 12A: Marking the gel with one hole respectively in three corners.

Fig. 12B: Scan (II) of the marked gel.



### 3.4 Scan (II) and storage of the gel

Scan the whole gel with the G-Dye 100 channel and a resolution of 100  $\mu\text{m}$  per pixel (Fig. 13). Export the image file without changing the format as tiff file. Make sure that the gel is humidified with Picking Solution (25% ethanol) during this. If possible, cover the gel until spot picking in order to avoid keratin contaminations.

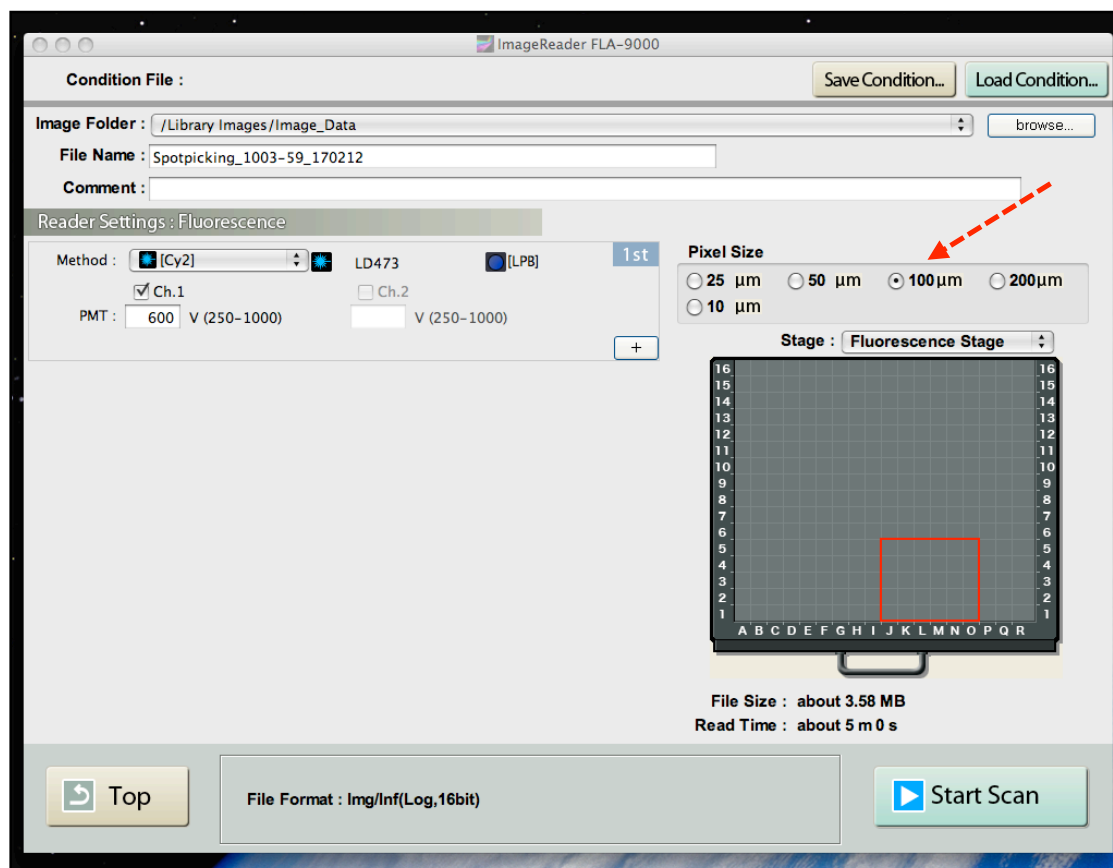


Fig. 13: Scanning parameters: Fujifilm FLA-9000 Image Reader (the red arrow marks the scanning resolution; screen shot)



### 3.5 Creating the picking template

Set the resolution of the tiff image with your image processing software (e.g. Adobe® Photoshop®) to 254 dpi (Fig. 14). Avoid resample of the image dimensions. Print the adapted tiff image and make sure that the printing scale is set to 100 % (Fig. 15).

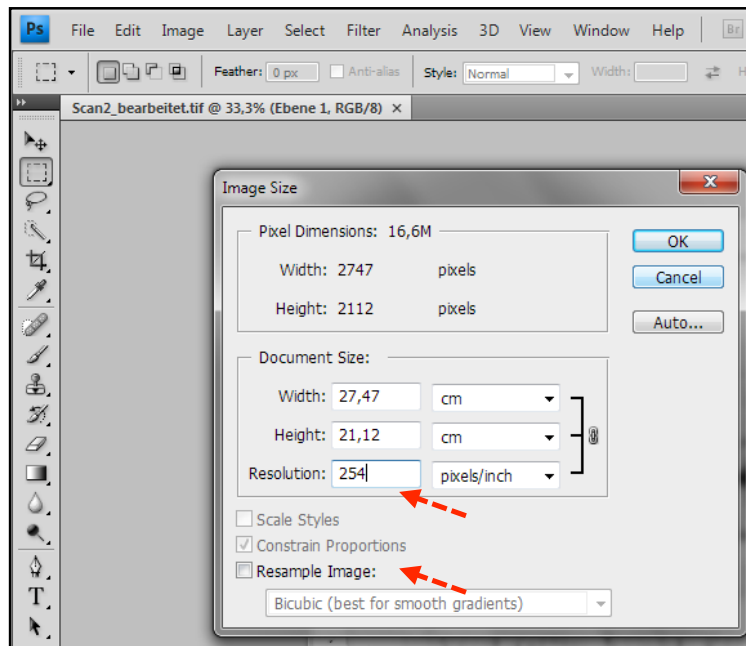


Fig. 14: Adaption of the resolution from the tiff image with Adobe® Photoshop® (screen shot).

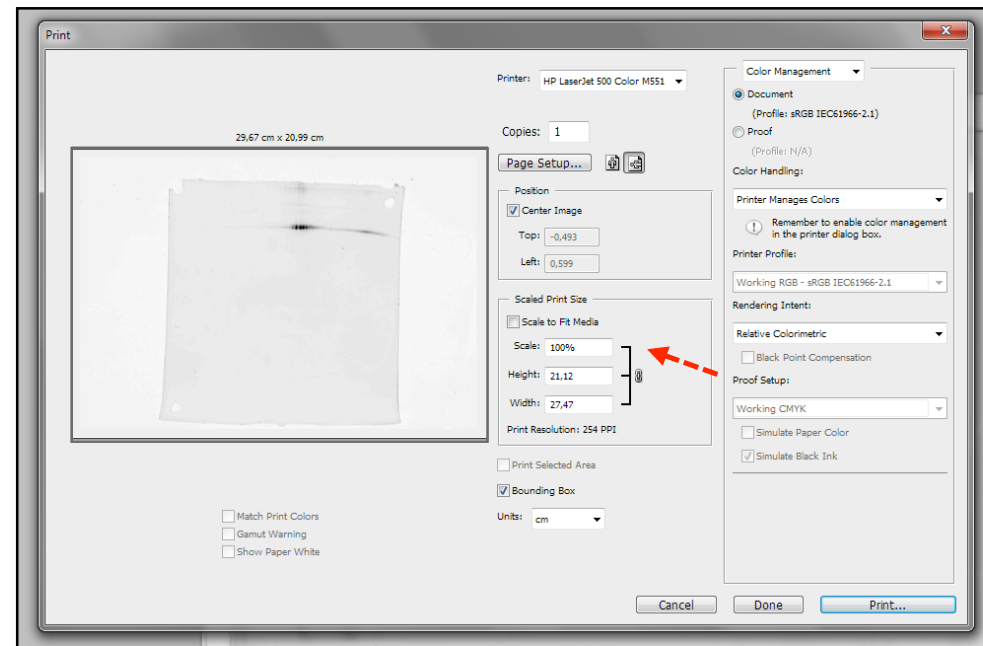


Fig. 15: Print settings (screen shot)

### 3.6 Marking of the picking template

Encircle the marks in the corners of the spot picking template in order to facilitate the matching of the gel and the printout.

### 3.7 Spot picking

Place the printout of the spot picking template under the low fluorescence glass plate with the gel on it. Adapt the position of the gel in order to perfectly match the positions of the marks in the gel with the encircled marks on the printout (Fig. 16). Pick now the BSA spots and transfer them into a 1,5 ml microcentrifuge tube.

As a control, scan your gel again with the G-Dye 100 channel. The BSA spots should not be detectable anymore. (Fig. 17). For further control you can stain your gel with Coomassie Blue or silver nitrate.

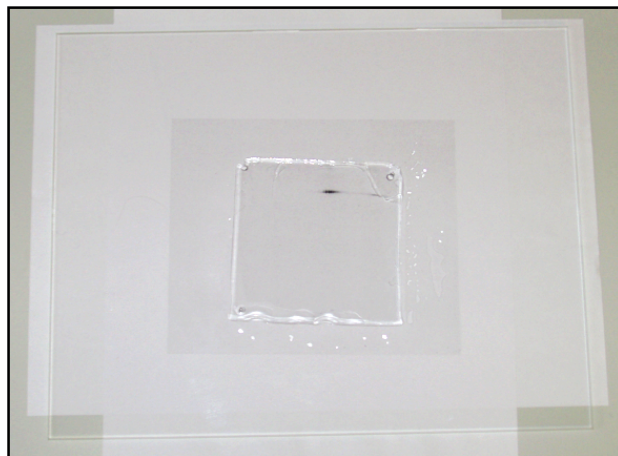


Fig. 16. Preparation for spot picking. The picking template is placed under the gel in the way to perfectly match the position of the gel marks with the encircled marks on the printout.

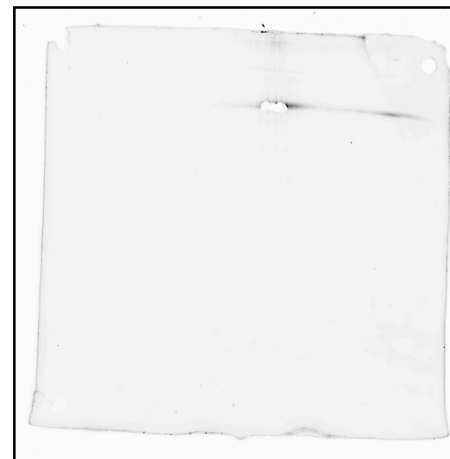


Fig. 17: G-Dye 100 image of the gel after spot picking.

## 4 Tips & Tricks

### 4.1 Marking of the gel

For marking the gel (see Quick Guide 2.2 and Detailed Guide 2.3.4) you can as well use other methods like cutting corners or other marking which help you to perfectly match your gel and the spot picking template.

### 4.2 Spot picking without low fluorescence glass plates

If you should not possess low fluorescence glass plates, you can as well use any other type of transparent glass or plastic plate for spot picking. In this case you have to perform scan (II) without this plate and place your gel prior to spot picking on the respective plate. We recommend use of low fluorescence glass plates in order to keep gel damaging by handling to a minimum.

### 4.3 Distortion of the gel

If you constantly humidify the gel with Storage Solution, the gel will keep its shape for at least 1 hr. If you observe during scan (II) or during creating the spot picking template a swelling or shrinking of the gel, the differences can be corrected as follows:

*(a) The gel is smaller than the picking template:*

Place the gel briefly in water and wet it on the glass plate to let it swell. If the gels reached the required size, start with spot picking.

*(b) The gel is larger than the picking template:*

Place the gel briefly in fixating solution. If the gel reached the required size, rinse briefly with Picking Solution and start with spot picking.

#### **4.4 Use of pre-casted gels**

The Refraction-2D™ Spot Picking Guide is compatible to all pre-cast gel systems, which are suited for spot picking (glass- or plastic cassettes have to be removable). Please consider as well the supplier's recommendations for storing and fixing of the gels.

Using foil supported gels, please consider the supplier's recommendations for fixating and storage of the gels. Foil supported gels are covalently attached to the foil. The gel cannot deform. Therefore you can use scan (I) for preparing the picking template and skip scan (II). Furthermore, you do not need to mark foil supported gels, since gel and foil margins are usable as marks. Make sure to scan with foil completely. For extended storage (e.g. evaluation of the gel between scan (I) and spot picking) we recommend the fixation and drying of the gel (please refer to the supplier's recommendations). Rehydrate the gel before spot picking.

## 4.5 Preparing preparative gels

For preparing preparative gels we recommend to use 100 µg of G-Dye 100 labeled proteins (the Refraction-2D™ Kit (≥12G) contains an extra aliquot of G-Dye 100 for preparative gels) as well as the spiking with non-labeled protein sample in order to increase the identification chance of low abundant proteins during MS. Please refer as well to the recommendations of your IEF strip supplier for the maximum amount of protein that can be loaded. Separate your preparative sample like your analytical samples by 2D gel electrophoresis as before.

*If you wish to pick spots immediately after 2D gel electrophoresis:*

Start with section 2.3.4 by placing your gel without fixation or rinsing on a low fluorescence glass plate, marking it, and scanning it (corresponds to scan (II)). Wet the gel with Picking Solution and continue as outlined in the Refraction-2D™ Spot Picking Detailed Guide.

*If you wish to pick spots later:*

Start with section 2.3.2 by scanning your gel as a control, subsequently fixate your gel, and keep it in Storage Solution until spot picking. Continue before spot picking as outlined in the Refraction-2D™ Spot Picking Detailed Guide section 2.3.4 and following sections.

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