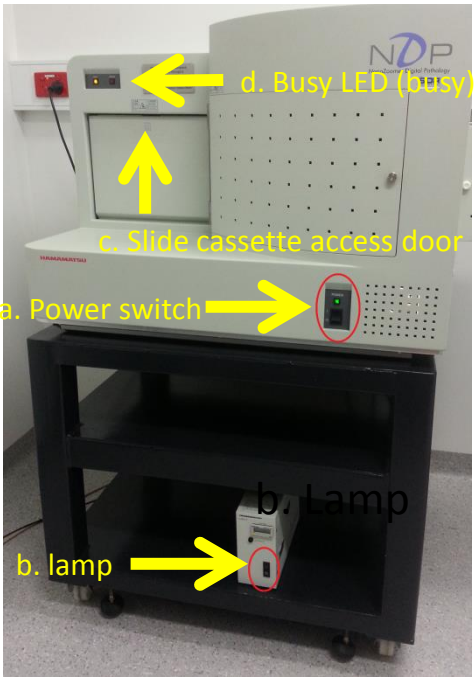
Brief how-to instruction on the NanoZoomer (NZ)

(prepared by Hong Yu 13 May 2016)

Start up

• Turn on the power for NZ (a) ⟶ mercury lamp (b) if needed ⟶ computer.
Warm up for 30 min



Loading slides

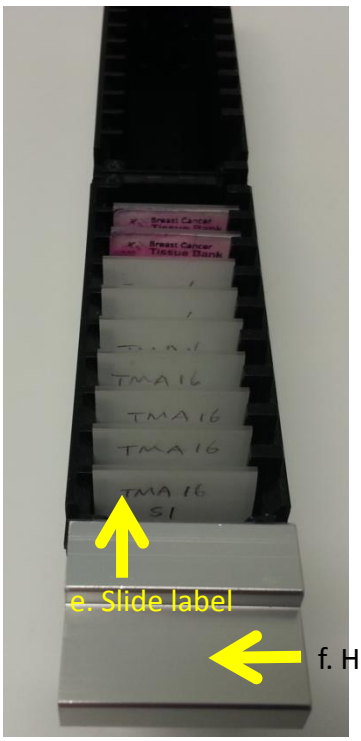
- Push to open the slide cassette access door (c).

Note: the door does not open when BUSY LAMP is on (orange) (d).

- Insert the slides to the slots with the sample surface upside and the labels (e) near the cassette handle (f).

Note: DO NOT set more than 2 slides into one slot
DO NOT set slide to span two slots

- up to 30 sides can be set into a slide cassette (7 cassettes available total 210 slides can be scanned in one go)

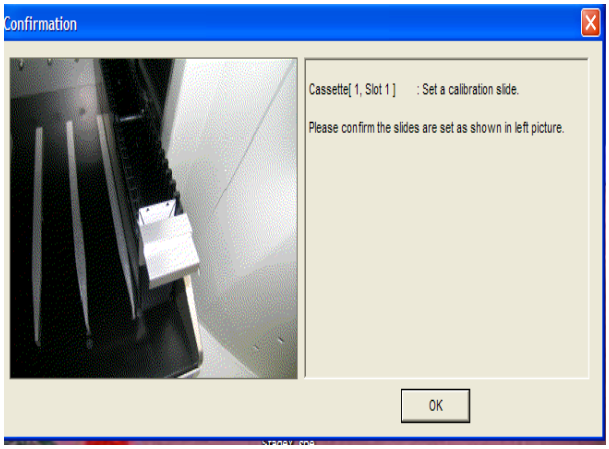


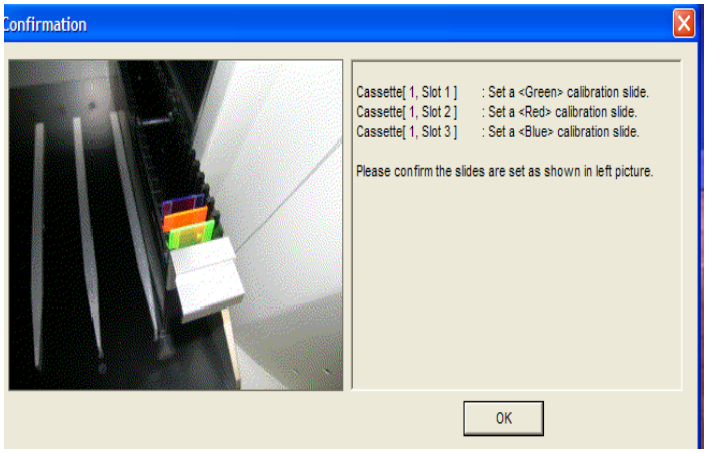
Slide preparation

- **NEVER** load slides into the NanoZoomer that are wet or have mounting medium that is not completely set.
- Check that the edges of slides are flush with their coverslips. If a coverslip is sticking out, or if there is a lump of mounting medium on any edges of a slide, use the sand paper to make the edge smooth.
- Make sure sticky labels do not stick out beyond the slide. Slice any edges that stick out with the razor blade.
- Clean the slides with 100 % ethanol before loading.

Calibration—BF and fluorescence calibration

load the calibration slide ⟶ click NDP calibration (BF/Fluo) on the desktop of the computer ⟶ press ok





Note:

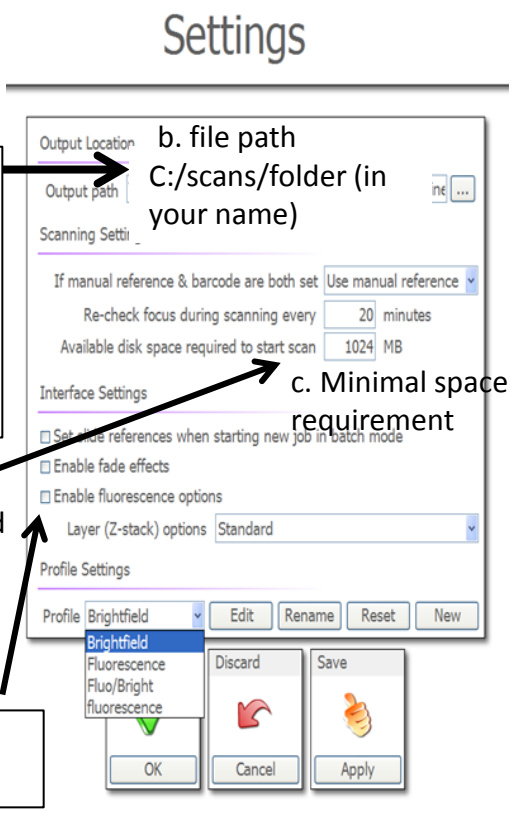
- The default fluorescence filter cube in position is the triple one (DAPI/FITI/TRITC).
- The fluorescence calibration slides have to be arranged in this particular order of colours as shown above (G-R-B).
- Fluorescence calibration: choose “triple” (only need to run calibration once).
- Handle the calibration slides with care, they are very expensive

Note: It’s recommended to do calibration every time before scanning slides, especially for fluorescent slides, it takes only 5 minutes.

Scanning and image acquisition

- Open NDP-scan 2.260 software (this will take a couple of minutes → load the slides
- Click "Settings" (a) in the software. Steps b-d.

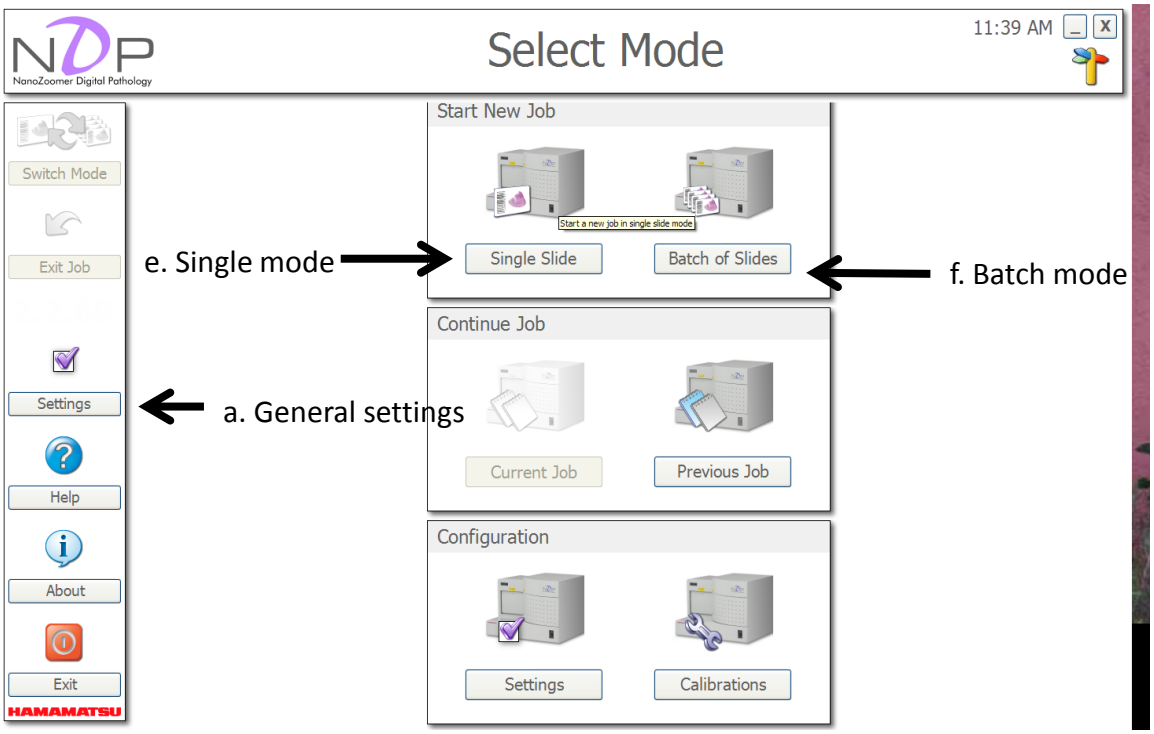
Temporarily put images to C:/Scans (b) in a subfolder in your name for maximum up to **TWO** weeks on the local computer → move it to the server or a portable hard drive afterwards.



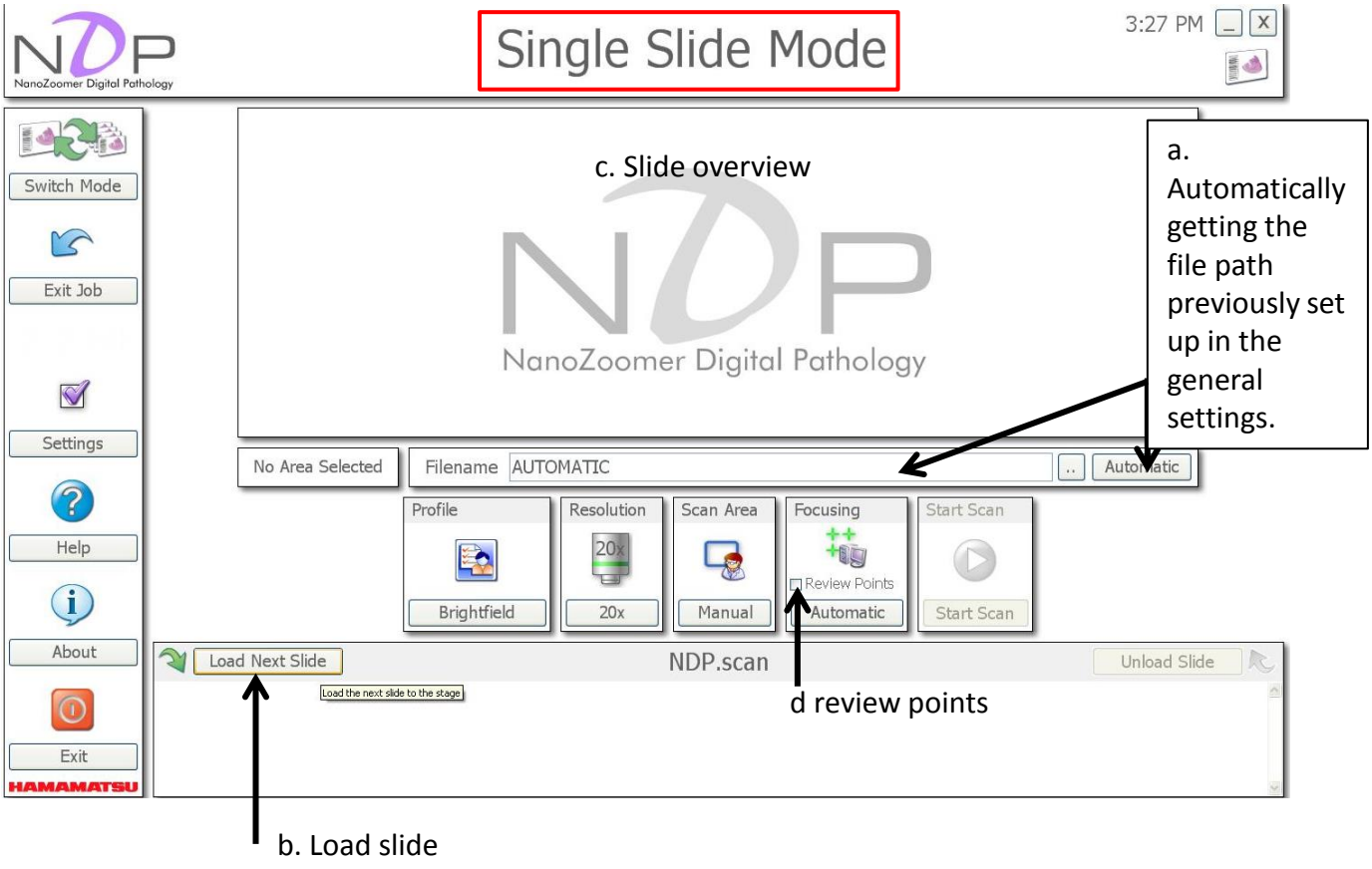
1024 MB (c) is recommended for space requirement.

Choose Profile, when doing fluorescence scanning, tick "**Enable fluorescence options"** (d).

- Choose "single" mode (e) if you want to scan individual slides one at a time. Or choose "batch" mode (f) if you want to scan a number of slides unattended (either automatically or with manual intervention). You can switch between these two modes when needed.



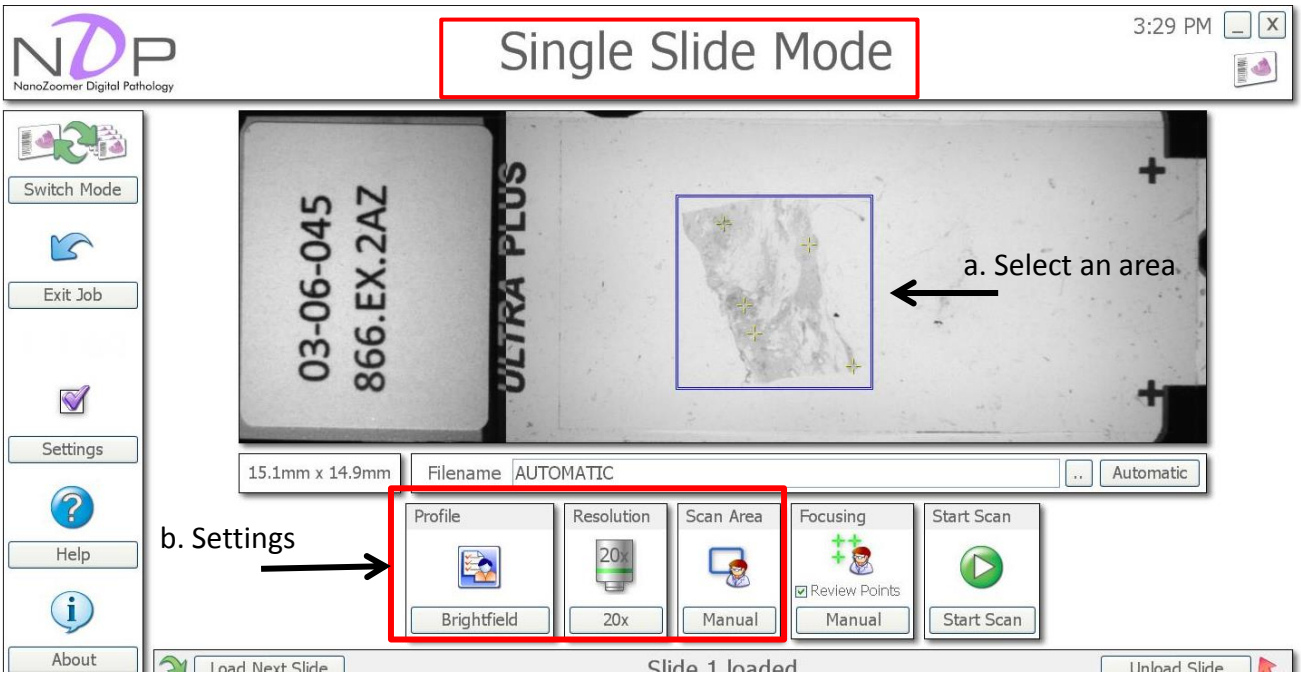
Single slide mode (BF)



- Click “automatic” (a) to get the right file path as set in the general settings (refer to page 3 of this manual).
- Click “load next slide” (b). You will see an overview of the slide in the live window (c).

Note: Compared to batch mode, the results if chosen “single slide mode” are usually better. In addition, the live window field to set focus points is larger and there is also an option to review your focus points, a small field will appear if you check the box “review points” (d) and you can zoom in or out. It’s more time consuming though.

Continued: single mode (BF)



- Select the speciman for scanning (a). The current software version does not allow selecting multiple areas.
- Go through the imaging settings (b) including:

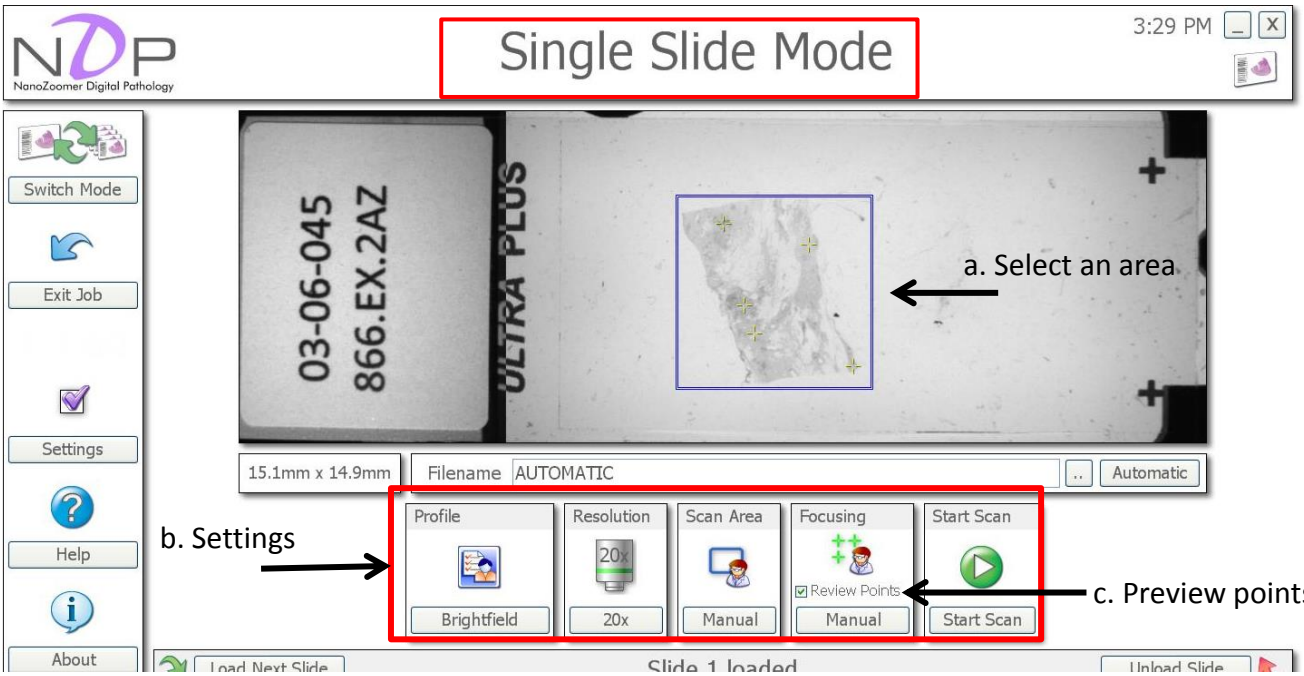
Profile: BF

Resolution: 20x or 40x (they are not objectie magnifications just names for 2 different resolution. 20X: 460 nm per pixel; 40X: 230 nm per pixel.

Scan area (a): automatic or manual. Usually automatic mode does a good job if samples are of good contrast. Manual mode allows you to freely select the area by drawing a blue box. to be scanned. **Focusing**: **RIGHT** click inside box to choose focus points

- Click “Start scanning” to process imaging

Continued: single mode (BF)



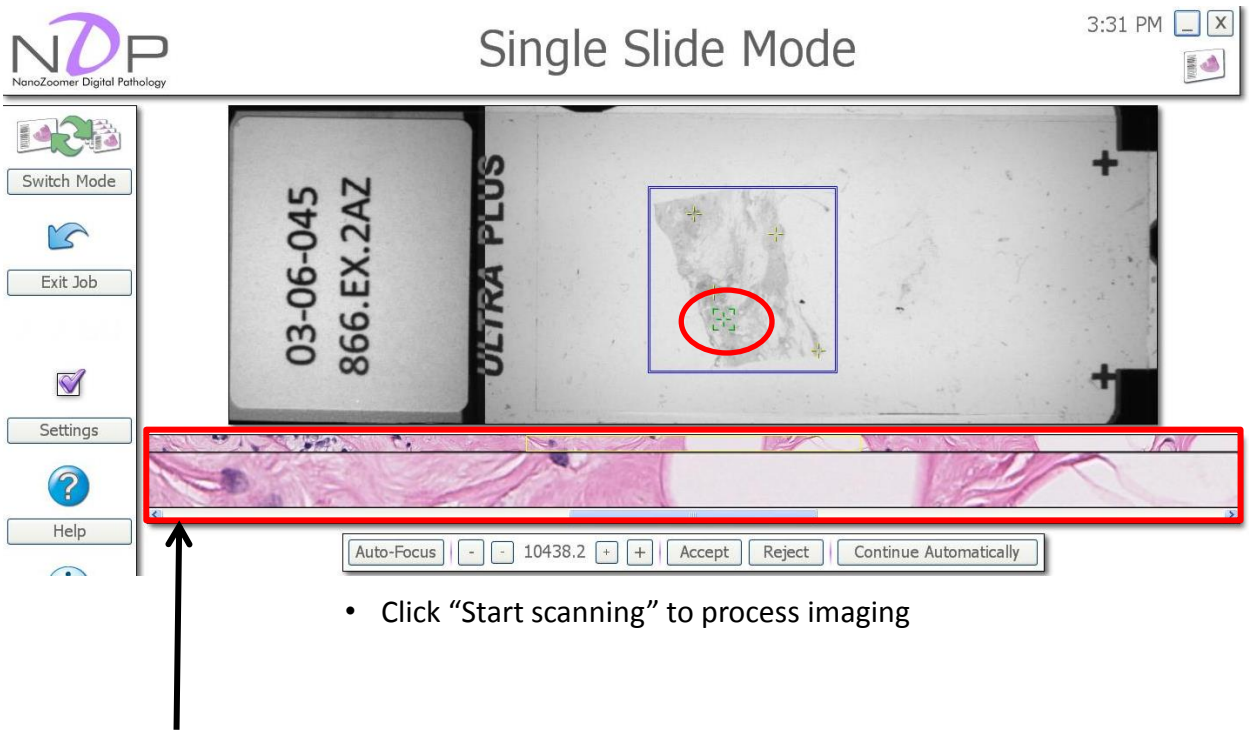
- Select the specimen for scanning (a). The current software version does not allow selecting multiple areas.
- Go through the imaging settings (b) including:
  Profile: BF

Resolution: 20x or 40x (they are not objective magnifications just names for 2 different resolution. 20X: 460 nm per pixel; 40X: 230 nm per pixel.

Scan area (a): automatic or manual. Usually automatic mode does a good job if samples are of good contrast. Manual mode allows you to freely select the area by drawing a blue box. to be scanned.

Focusing: automatic or manual. If you choose manual, **RIGHT** click inside box to choose focus points. When check the "preview points" box (c), you will get a window to review the focusing points (a in the next image—next page).

Continued: single mode (BF)



- Click “Start scanning” to process imaging

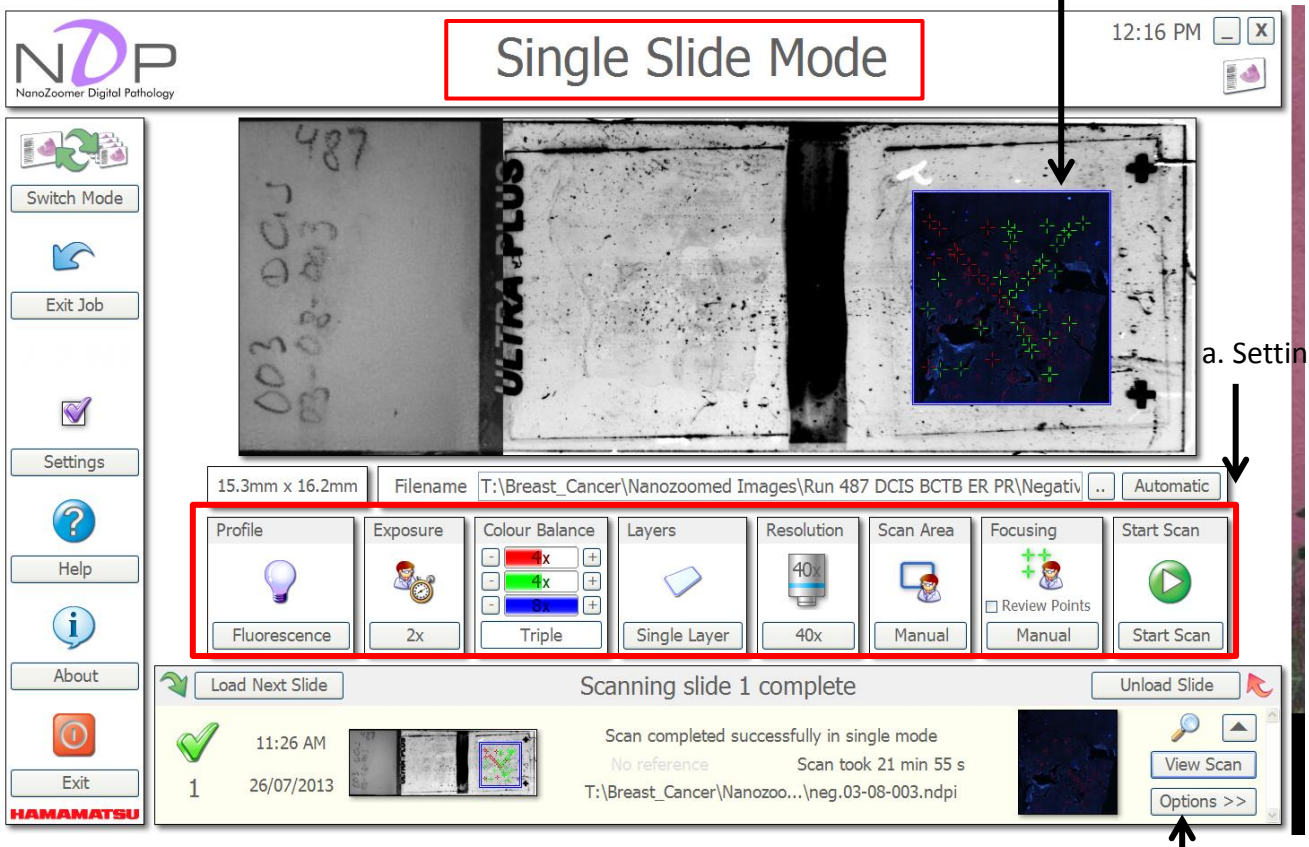
d. Preview of the current focusing point (green cross with a red circle in the live window)
Top: The full length image from the line scanner
Middle: full size camera image inside the highlighted yellow box in the image above
Bottom: moving the slide will let you scroll through the line scanner image

Continued: single mode (Fluo)

- Go through the imaging settings (a) including:
  Profile: Fluo or BF

  Exposure and color balance: For Fluo scanning, the conditions of "exposure x2, color balance x4 for red and green and x8 for blue" usually give you a good result or at least are a good start to try
  Layers: Single or multiple layers
  Exposure : either 20x or 40x
  Scan area: select the area to be imaged by drawing a blue box
  Focusing: **RIGHT** click inside box to choose focus points if Manual
- Click "Start scanning" to process imaging

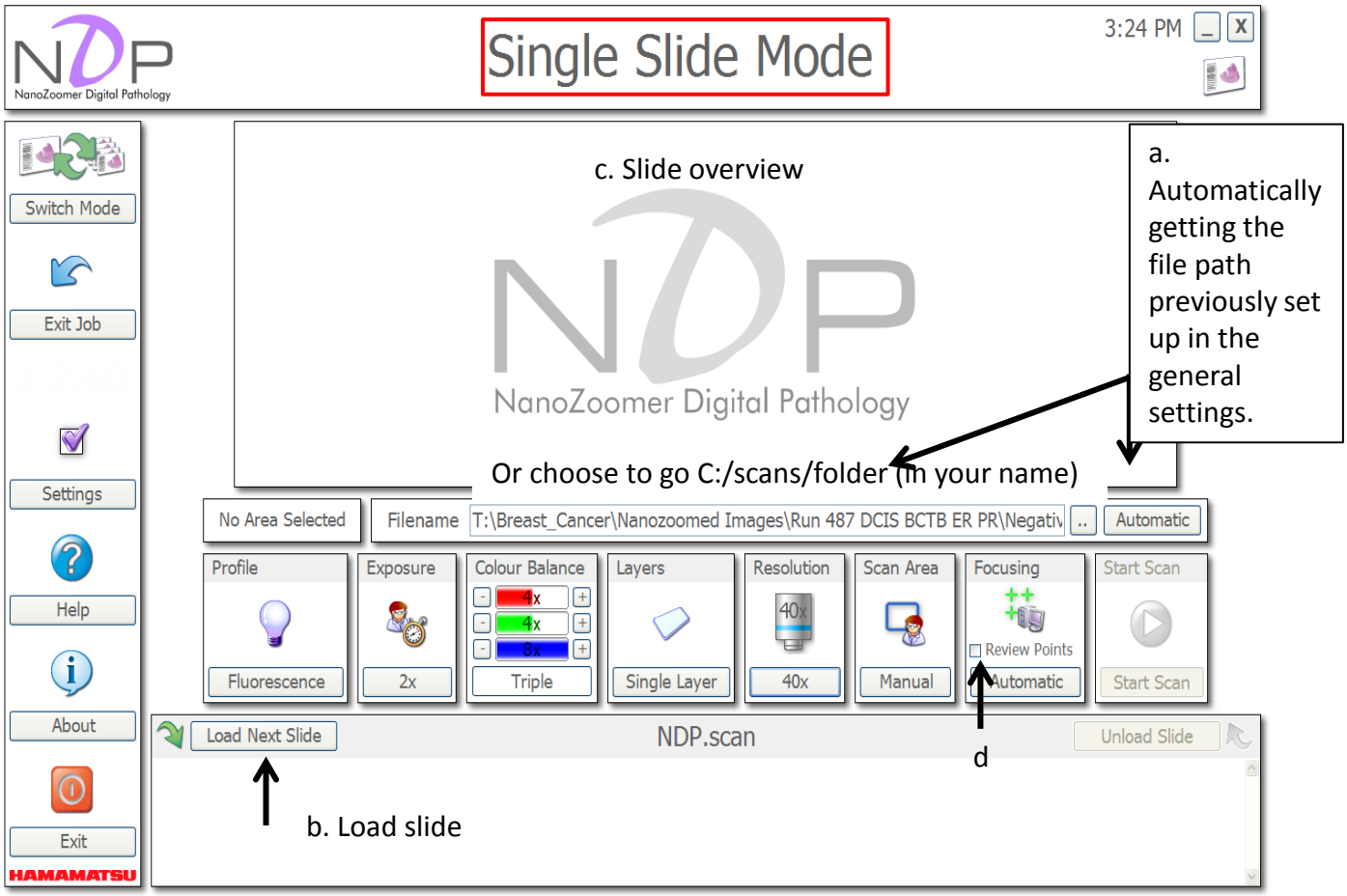
This sample shows which focus points (b) could not be detected by the machine (red cross) (b).



Tip: Sometimes it is difficult to see the actual sample/area of interest in the Fluorescence modus, you can draw a circle around the specimen using a Sharpie pen before scanning.

Use **options** to reject scan if preview shows a unsatisfactory image

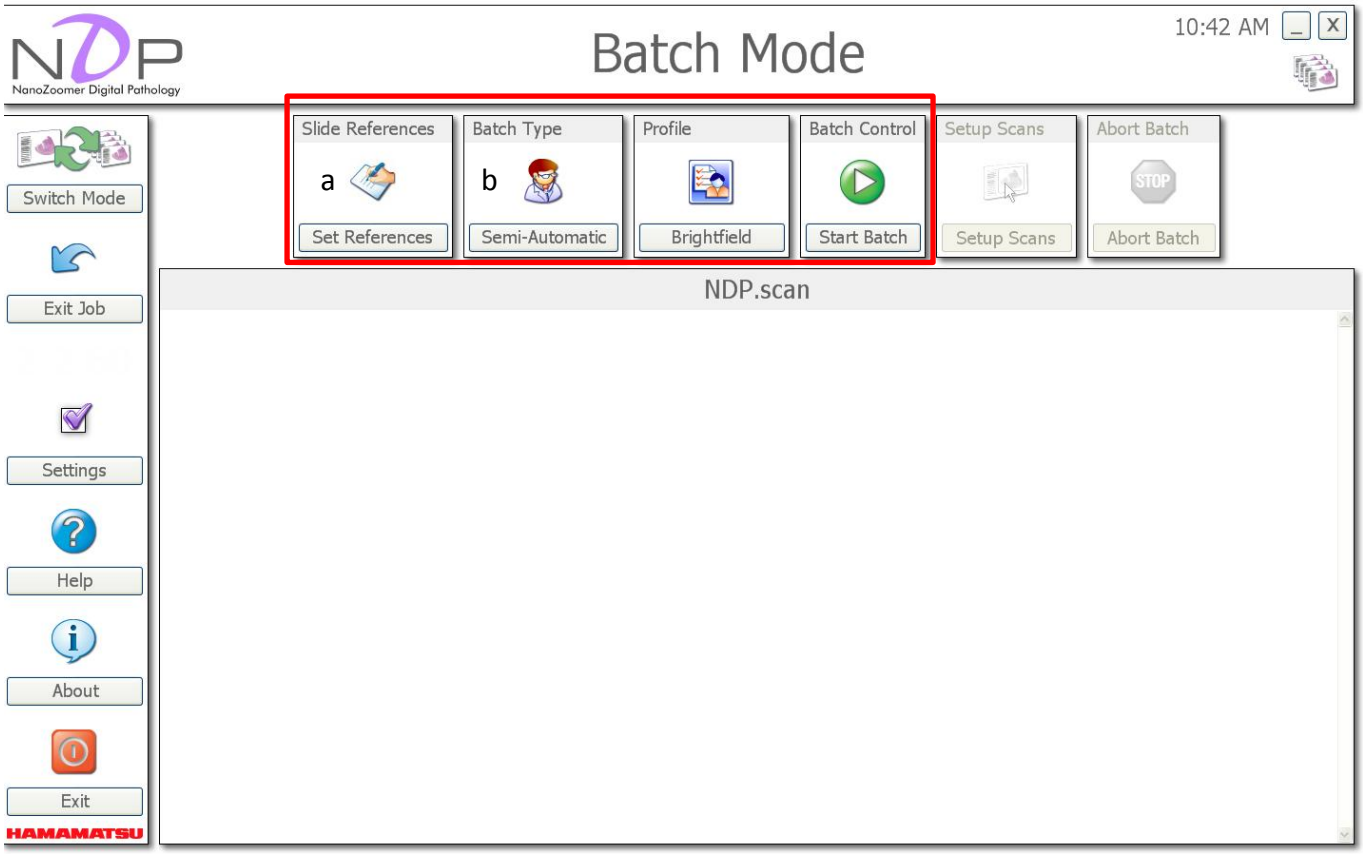
Single slide mode (Fluo)



- Click “automatic” (a) to get the right file path as set in the general settings (refer to page 3 of this manual).
- Click “load next slide” (b). You will see an overview of the slide in the live window (c).

Note: Compared to batch mode, the results if chosen “single slide mode” are usually better. In addition, the live window field to set focus points is larger and there is also an option to review your focus points, a small field will appear if you check the box “review points” (d) and you can zoom in or out. It’s more time consuming though.

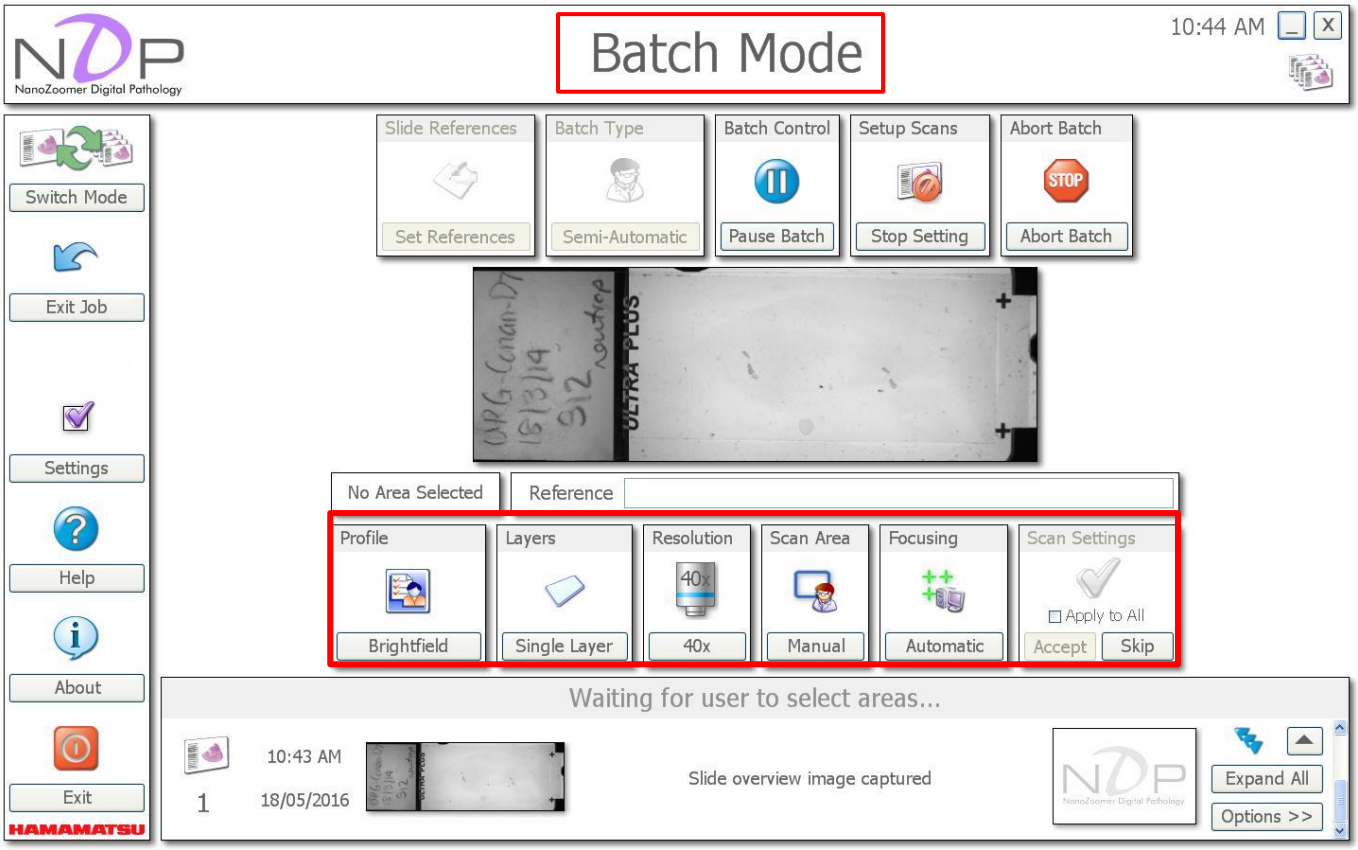
Batch mode (BF)



- Set Reference (a): name your slides—you can also do this step at the earlier stage of “Settings”.
- “Automatic” is not recommended, choose “semi-automatic” (b).
- Profile (c): should display “brightfield” if you chose it earlier when setting “settings
- Click “start batch” to load the slides. After this, a window will pop up with the preview image of the fist slide (see below image)

- Click “set up scan” (d)

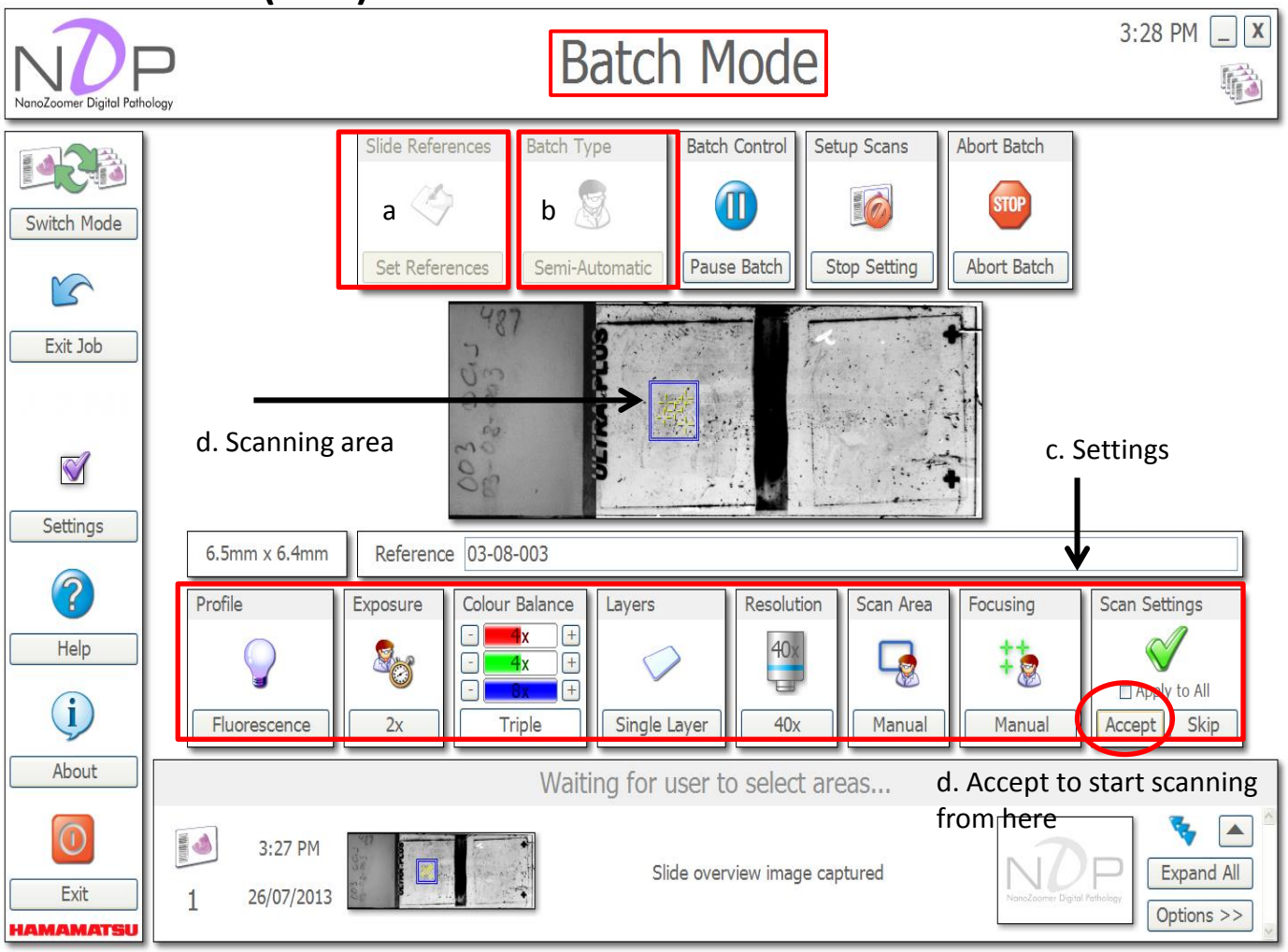
Batch mode (BF)--continued



- **Profile**: Brightfield
- **Layers: single or different options**
- **Resolution:** 20x or 40x (they are not objective magnifications just names for 2 different resolution. 20X: 460 nm per pixel; 40X: 230 nm per pixel.
- **Scan area (a)**: automatic or manual. Usually automatic mode does a good job if samples are of good contrast. Manual mode allows you to freely select the area by drawing a blue box to be scanned.
- **Focusing**: automatic or manual. If you choose manual, **RIGHT** click inside box to choose focus points. When check the "preview points" box (c), you will get a window to review the focusing points (refer to Slide 7).
- Finally click "Accept" to process to set up next slide and initialize scanning.
- Once all slides are scanned, click "exit job" to unload the slides.

Tip: You can choose to stop setting or abort batch at any stage (above the slide preview window).

Batch mode (Fluo)



- Set Reference (a): name your slides—you can save your reference file or load your previous file.
- "Automatic" is not recommended, choose "semi-automatic" (b).
- Go through "settings (c) including

  Profile: Fluo or BF

  Exposure and color balance: For Fluo scanning, the conditions of "exposure x2, color balance x4 for red and green and x8 for blue" usually give you a good result or at least are a good start to try

  Layers: Single or multiple layers.

  Exposure : either 20x or 40x

  Scan area: select the area to be imaged by drawing a blue box

  Focusing: **RIGHT** click inside box to choose focus points

- Click "Accept" (d) to process imaging

Tip: The smaller the area to be scanned (size of the blue box, e), the better the results usually are

Shut down

- Close software and shut down computer
- Remove your slides from the Nanozoomer and return racks to the storage shelf
- Turn off the fluorescence lamp
- Turn off the Nanozoomer
- Clean the bench
- Log your usage
- Leave room tidy

Other notes

- The 16 bit color camera works in as a letter box stripe/lane mode for scanning (width 800um. I.e. scanning width is 10.3X1.6mm, then lanes=10.3/0.8mm=13 lanes).
- IF you see stripe in your image, you should run calibration. Minimal once a week for BF and every time for Fluo.
- do not forget to turn off “review points” in the focusing setting. Otherwise, it will do the same thing to the next slide.
- BF calibration does white balance as well
- when save image at 20X, resolution is better but you lose scaling (scale bar looks strange). This is an issue in NDP viewer 2.
- unable to do BF and Fluo in one scanning. Higher software version can solve this problem.
- Mercury lamp cooling time should be over 10 min. Warm up takes about 30 min.

Report issues to Dr Hong Yu

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