

Brief how-to instruction on the NanoZoomer (NZ)

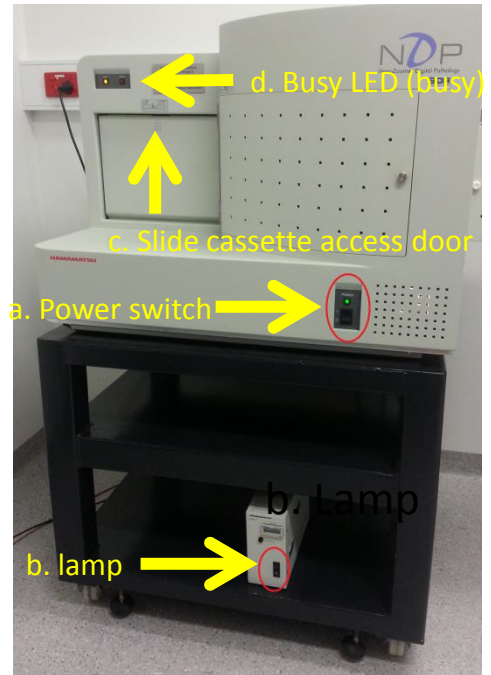
(prepared by Hong Yu 13 May 2016)

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)



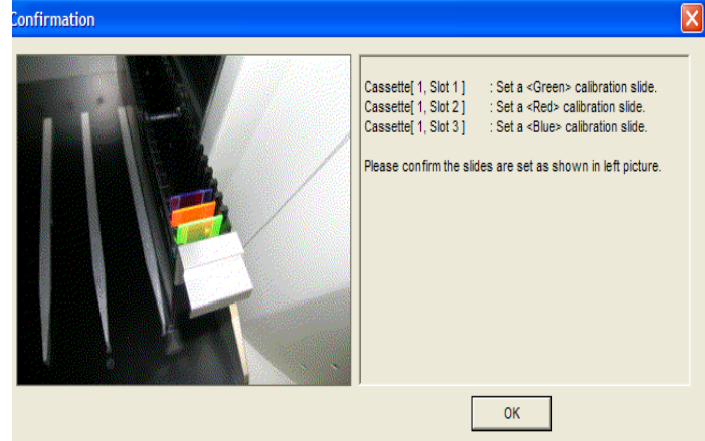
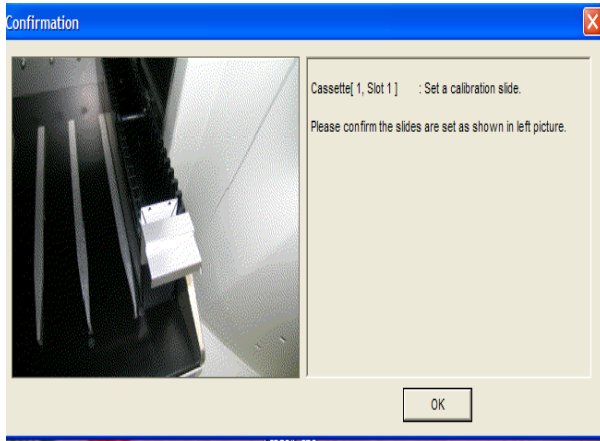
Slide preparation

- **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.

f. Handle (silver part)

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/FIT1/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).

Settings

The screenshot shows the 'Settings' dialog box with the following sections and annotations:

- Output Location:** Output path is set to 'C:/scans/folder (in your name)'. An arrow points to this field with the label 'b. file path'.
- Scanning Settings:** 'Available disk space required to start scan' is set to '1024 MB'. An arrow points to this field with the label 'c. Minimal space requirement'.
- Interface Settings:** 'Enable fluorescence options' is checked. An arrow points to this checkbox with the label 'd'.
- Profile Settings:** The 'Profile' dropdown is set to 'Brightfield'. A dropdown menu is open showing 'Fluorescence' and 'Fluo/Bright fluorescence' as options.

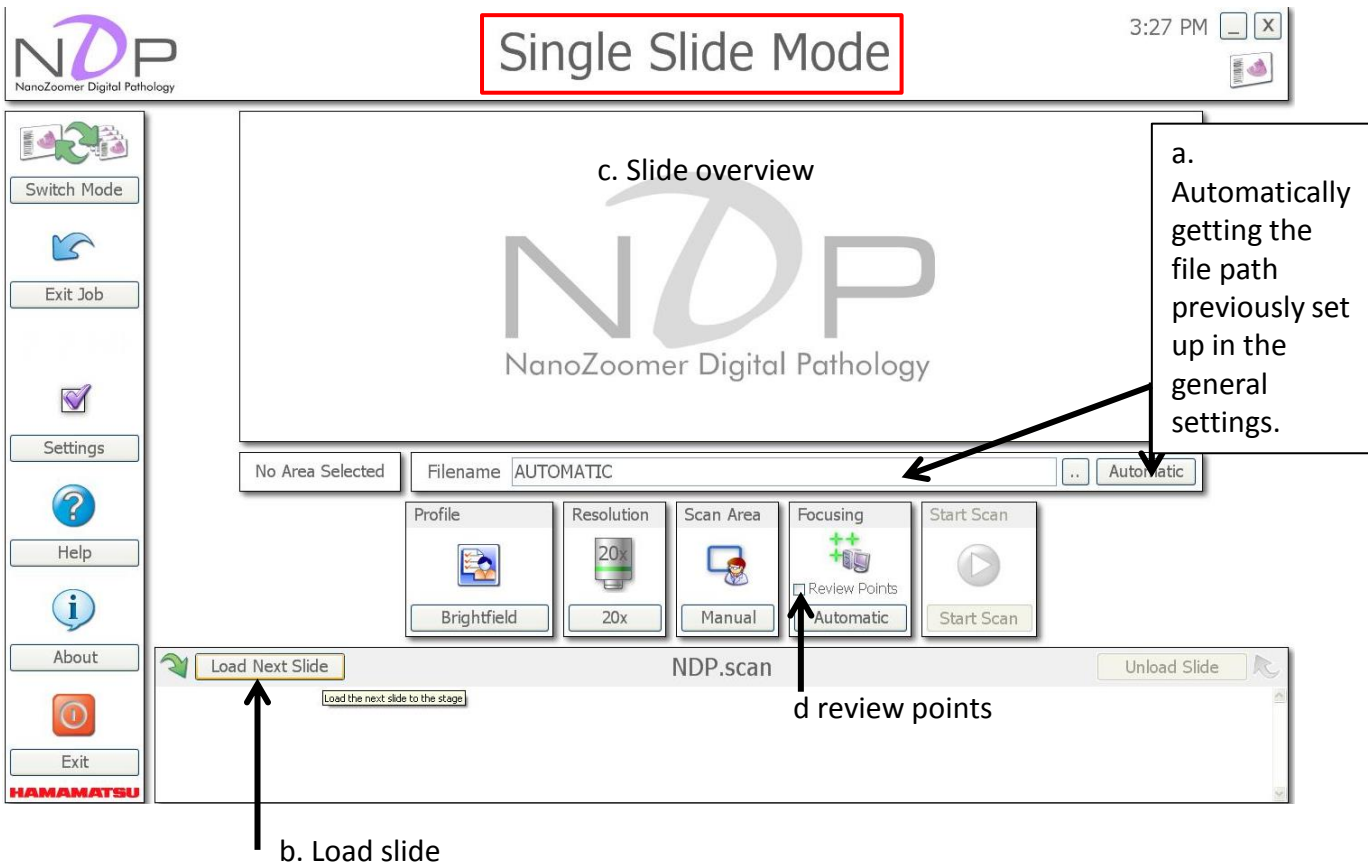
- Choose “single” mode (e) if you want to scan individual slides one at a time. Or choose “batch” mode (f) if you want to scan a number of slides unattended (either automatically or with manual intervention). You can switch between these two modes when needed.

The screenshot shows the 'Select Mode' dialog box with the following sections and annotations:

- Start New Job:** Contains 'Single Slide' and 'Batch of Slides' buttons. An arrow points to 'Single Slide' with the label 'e. Single mode'. Another arrow points to 'Batch of Slides' with the label 'f. Batch mode'.
- Continue Job:** Contains 'Current Job' and 'Previous Job' buttons.
- Configuration:** Contains 'Settings' and 'Calibrations' buttons. An arrow points to 'Settings' with the label 'a. General settings'.

The dialog box also features a sidebar on the left with buttons for 'Switch Mode', 'Exit Job', 'Settings', 'Help', 'About', and 'Exit'. The top of the dialog shows the 'NDP NanoZoomer Digital Pathology' logo and the time '11:39 AM'.

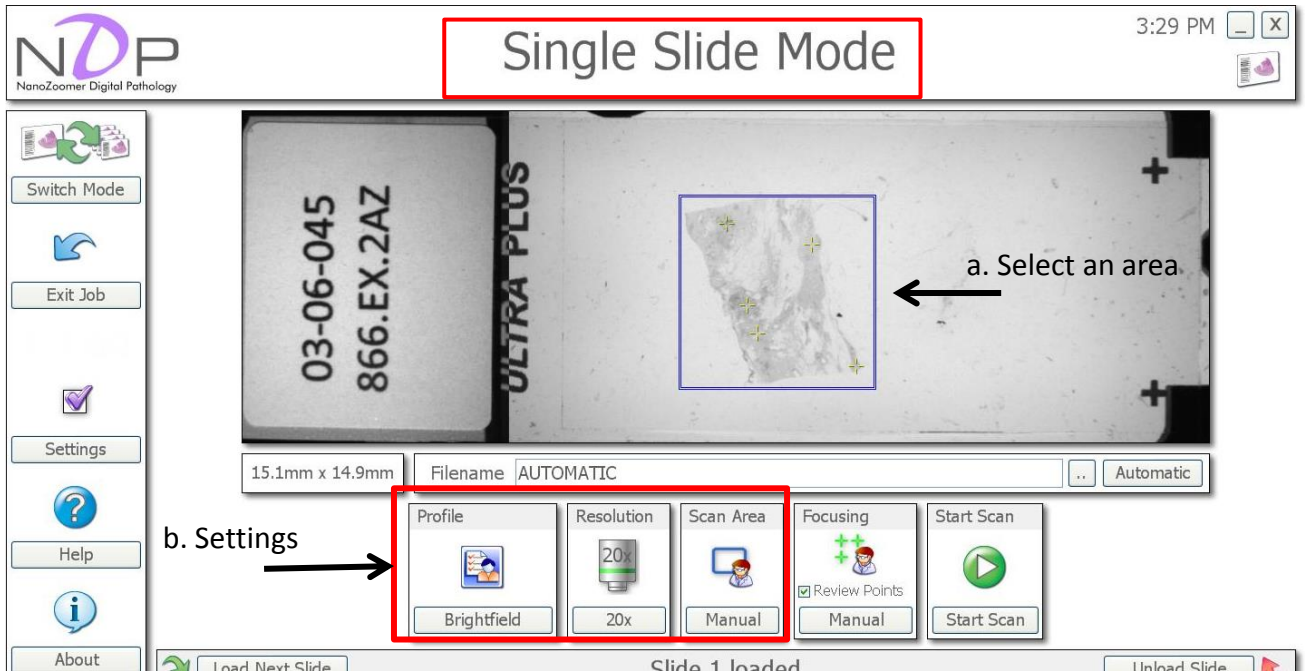
Single slide mode (BF)



- 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.

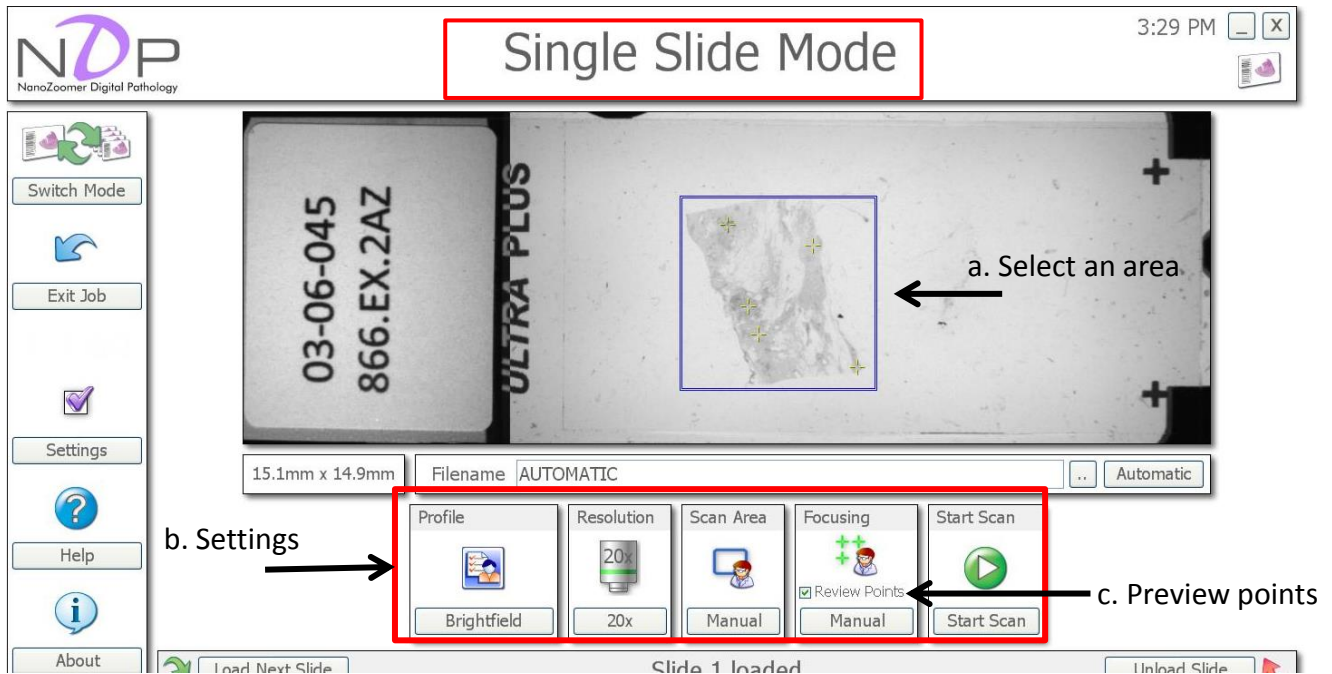
Continued: single mode (BF)



- Select the 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:** RIGHT click inside box to choose focus points

- Click "Start scanning" to process imaging

Continued: single mode (BF)



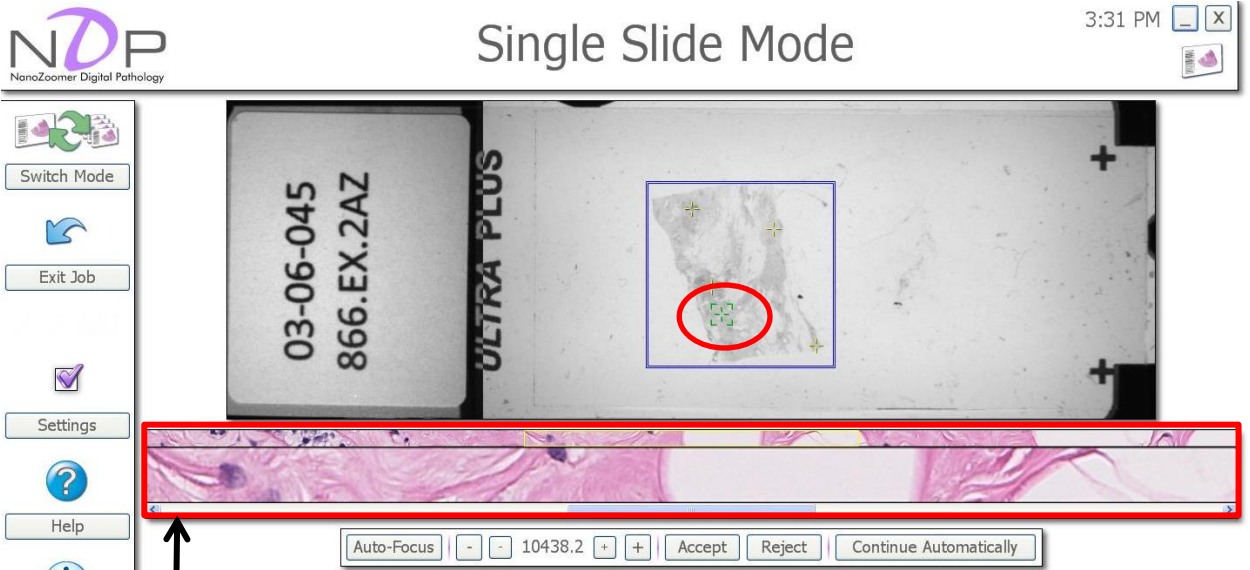
- Select the specimen for scanning (a). The current software version does not allow selecting multiple areas.
- Go through the imaging settings (b) including:
Profile: BF

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).

The screenshot displays the NanoZoomer Digital Pathology software interface in "Single Slide Mode". The main window shows a slide image with a blue scan area and focus points. A red box highlights the settings panel, which includes options for Profile (Fluorescence), Exposure (2x), Colour Balance (Triple), Layers (Single Layer), Resolution (40x), Scan Area (Manual), Focusing (Manual), and Start Scan. A red cross indicates a focus point that could not be detected by the machine. Arrows point to the settings panel and the "Options >>" button.

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)

NDP NanoZoomer Digital Pathology

Single Slide Mode

3:24 PM

c. Slide overview

NDP NanoZoomer Digital Pathology

Or choose to go C:/scans/folder (in your name)

No Area Selected | Filename: T:\Breast_Cancer\Nanozoomed Images\Run 487 DCIS BCTB ER PR\Negativ .. | Automatic

Profile: Fluorescence | Exposure: 2x | Colour Balance: Triple | Layers: Single Layer | Resolution: 40x | Scan Area: Manual | Focusing: Automatic | Start Scan

Load Next Slide | NDP.scan | Unload Slide

b. Load slide

d

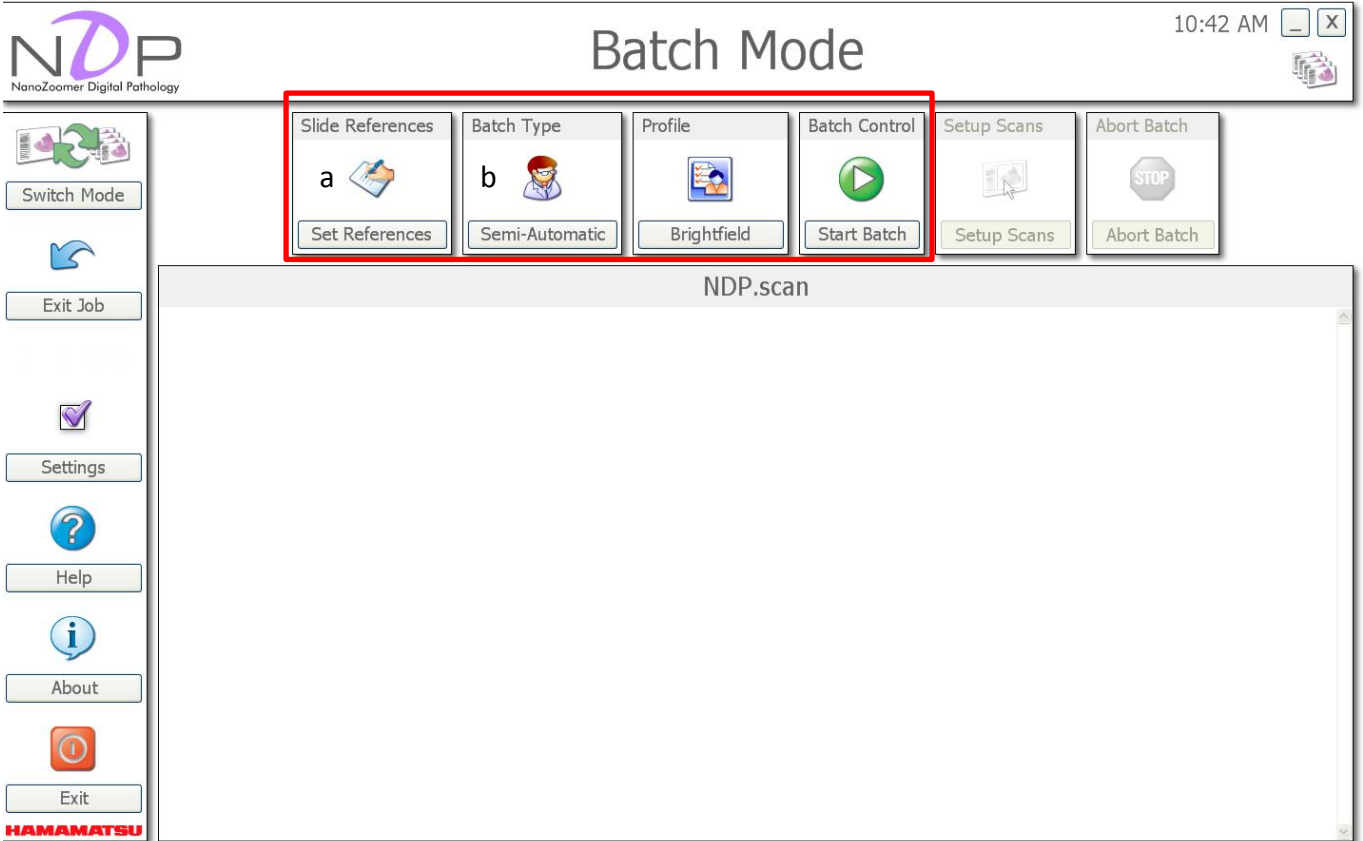
a. Automatically getting the file path previously set up in the general settings.

HAMAMATSU

- 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 first slide (see below image)



- Click “set up scan” (d)

d

Batch mode (BF)--continued

The screenshot displays the NanoZoomer Digital Pathology software interface in Batch Mode. The window title is "Batch Mode" and the time is 10:44 AM. The interface includes a left sidebar with buttons for "Switch Mode", "Exit Job", "Settings", "Help", "About", and "Exit". The main area shows a slide preview with handwritten text "VPG-Corona-D7 15/3/14 S12 newstrip ULTRA PLUS". Below the slide is a configuration panel with tabs for "Profile", "Layers", "Resolution", "Scan Area", "Focusing", and "Scan Settings". The "Profile" tab is selected, showing "Brightfield". The "Resolution" tab shows "40x". The "Scan Area" tab shows "Manual". The "Focusing" tab shows "Automatic". The "Scan Settings" tab has an "Apply to All" checkbox and "Accept" and "Skip" buttons. A status bar at the bottom indicates "Waiting for user to select areas..." and shows a slide overview image captured at 10:43 AM on 18/05/2016. The HAMAMATSU logo is visible in the bottom left corner.

- **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)



Switch Mode



Exit Job



Settings



Help



About



Exit

HAMAMATSU

Slide References

a

Set References

Batch Type

b

Semi-Automatic

Batch Control



Pause Batch

Setup Scans



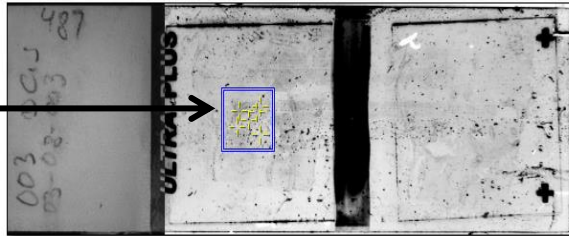
Stop Setting

Abort Batch



Abort Batch

d. Scanning area



c. Settings

6.5mm x 6.4mm Reference 03-08-003

Profile Fluorescence	Exposure 2x	Colour Balance Triple 4x 4x 8x	Layers Single Layer	Resolution 40x	Scan Area Manual	Focusing Manual	Scan Settings Apply to All Accept Skip
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Waiting for user to select areas...

d. Accept to start scanning from here

1 3:27 PM 26/07/2013

Slide overview image captured

NDP NanoZoomer Digital Pathology

Expand All Options >>

- 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= $10.3/0.8\text{mm}=13$ 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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