Advances in slide scanning technology

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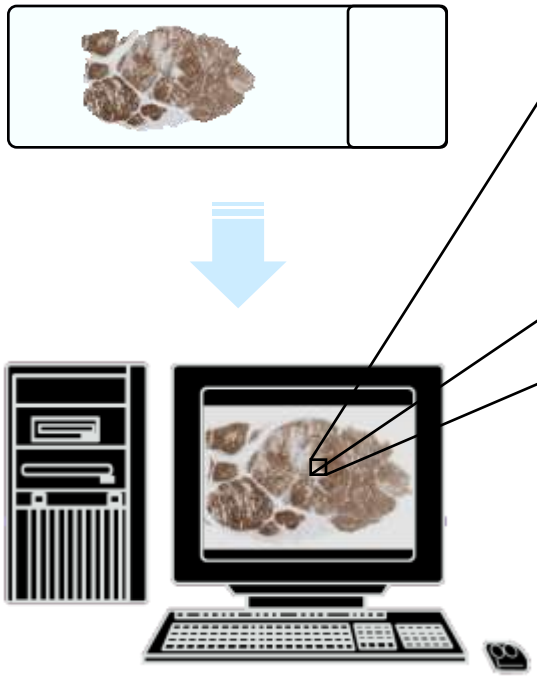
Agenda

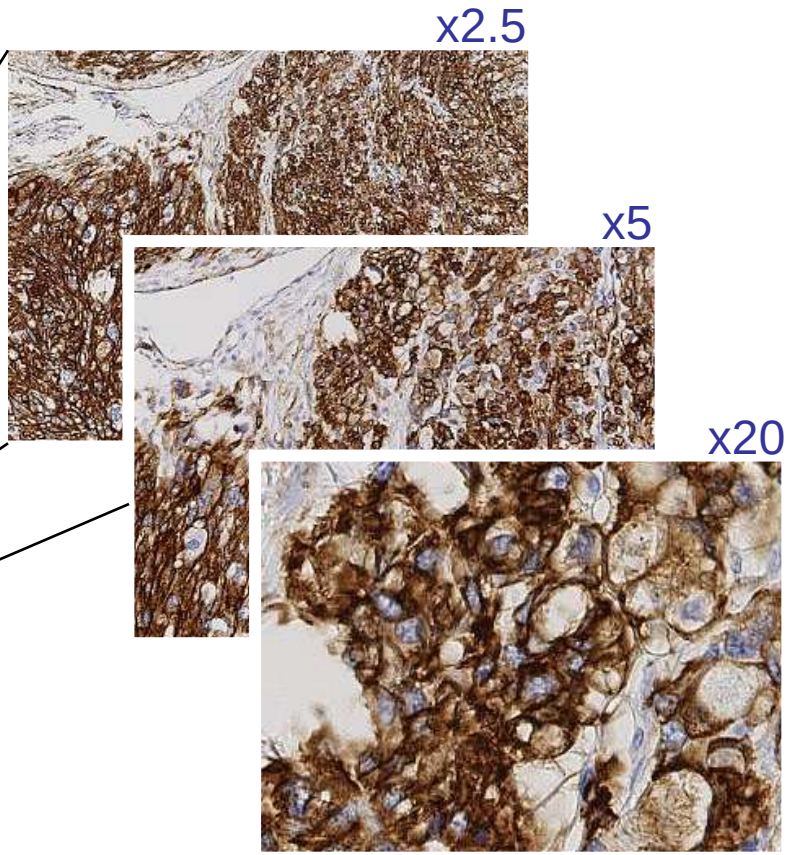
- History of “Virtual Microscopy”
- Scanning technologies
  - Magnification/Resolution/Pixel
  - Tissue recognition and focusing
  - Digitization method
  - TDI sensor scanning
- Advanced features
  - Fluorescence scanning
  - 3D stack

Virtual Slide

NDP
NanoZoomer Digital Pathology

High resolution scan of glass slides and storing digital slides into a PC.





Observation of any position at desired magnification on a monitor display

History of Virtual Microscopy

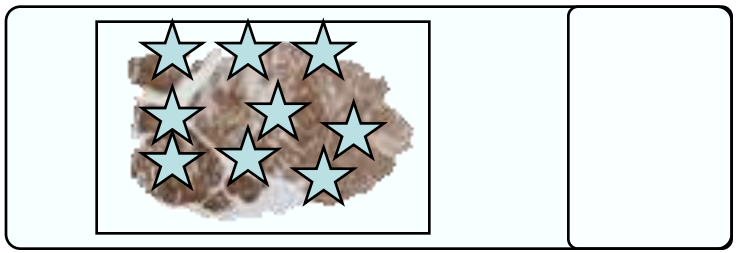
- Virtual Microscope concept was published in 1997 by computer scientists and pathologists.
  - This concept realized a computer based microscope through a display monitor by digitizing entire glass slides at the highest resolution required.
  - It required long time digitization and huge amount of storage area for the digitized data. It was limited to the concept for computer research, and it waited the computer technology and imaging technology advances to expand the concept for routine uses.
- Expansion of Virtual microscopy aided by the advances of computer power, digital storage, Internet access speed and Imaging technology
  - First Virtual Microscopy symposium was held at South Carolina University in 2002.
  - Commercial products were becoming available for various applications such as education, archiving and analysis.
  - In Japan, virtual microscopy is getting very popular in hospitals due to the government financial support for purchasing such systems for cancer diagnosis consultations.
- Standardization
  - DICOM Working Group (WG26) was established to create a standard of “Whole Slide Imaging (WSI)” to fit the virtual microscope into PACS system.

Magnification/Resolution/Pixel

- Magnification of Light Microscope
  - The magnification is defined as a product of magnifications of an objective lens and an eyepiece. It is a rough indication of view size of sample and optical resolution.
- Magnification of Virtual Microscope
  - The magnification is flexible, and it relates to a display monitor for viewing. The bigger the monitor size is, the higher the magnification is. The more pixels the monitor has, the lower the magnification is. The magnification is also relates to the pixel size of digitization. The smaller pixel the virtual slide has, the lower the magnification is.
    - The magnification is about 550 at
      - 20 inch monitor with 1600 X 1200 pixels
      - 0.46 µm pixel size of digitization
- Resolution
  - The optical resolution of a light microscope is defined by the numerical aperture (NA) of an objective lens. If the NA is 0.75, the resolution is approximately 0.4 µm.
  - To maintain the optical resolution, the pixel size of digitization needs to be smaller the optical resolution. However, reducing the size of pixel increases the data size. It is popular to have two levels of pixel size to be chosen by user preference such as 0.46 µm and 0.23 µm.
  - The number of pixels at 0.46 µm pixels size for 20mm X 20mm sample is about 1.9 billion. This can be compressed, and 2000 to 3000 slides can be stored into one tera byte hard disk. If the pixel size is 0.23 µm, the number of pixel is quadruple, and it requires 4 times more disc space.

Tissue recognition and focusing

- Tissue recognition and focusing are necessary features for automated virtual slide creation.
  - Entire glass slide image is captured by a CCD camera with a macro optics, and the image is analyzed to determine the area of sample.
  - Focus points are determined form the sample area, and the focus points are pre-scanned to determine each focus level. Based on the focus level, the focus of the entire sample is estimated.



Digitization method

- There is no cameras available to capture entire tissue sections at required microscope resolution.
- It is popular to use 2 dimensional CCD cameras or line scan sensors to scan tissue sections.
- Scanned images are stored as pieces of images as components of a virtual slide or as one large image.

Sensors for Virtual slide scanners

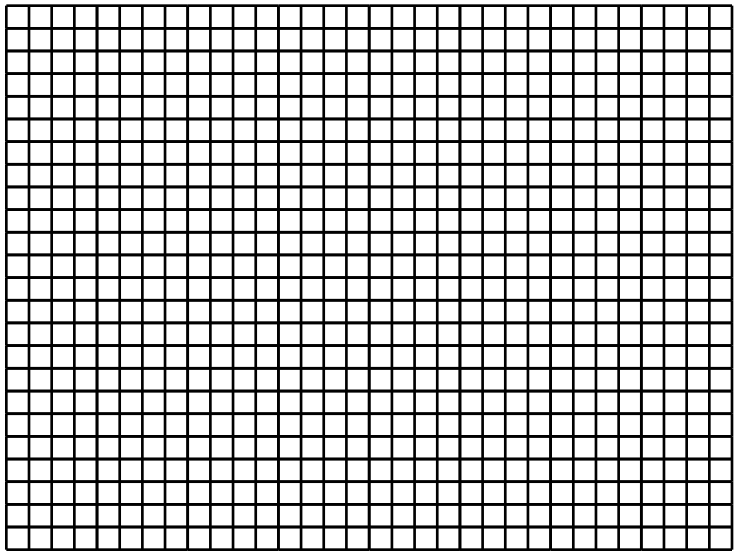
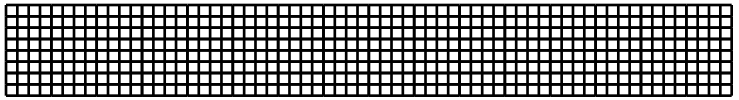


Image sensor (CCD)

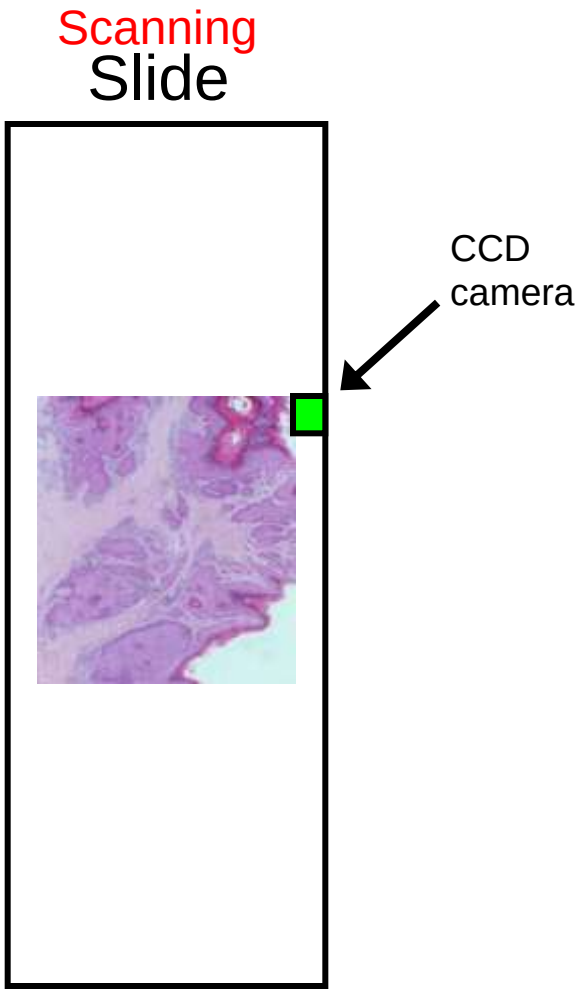
Line sensor (CCD)



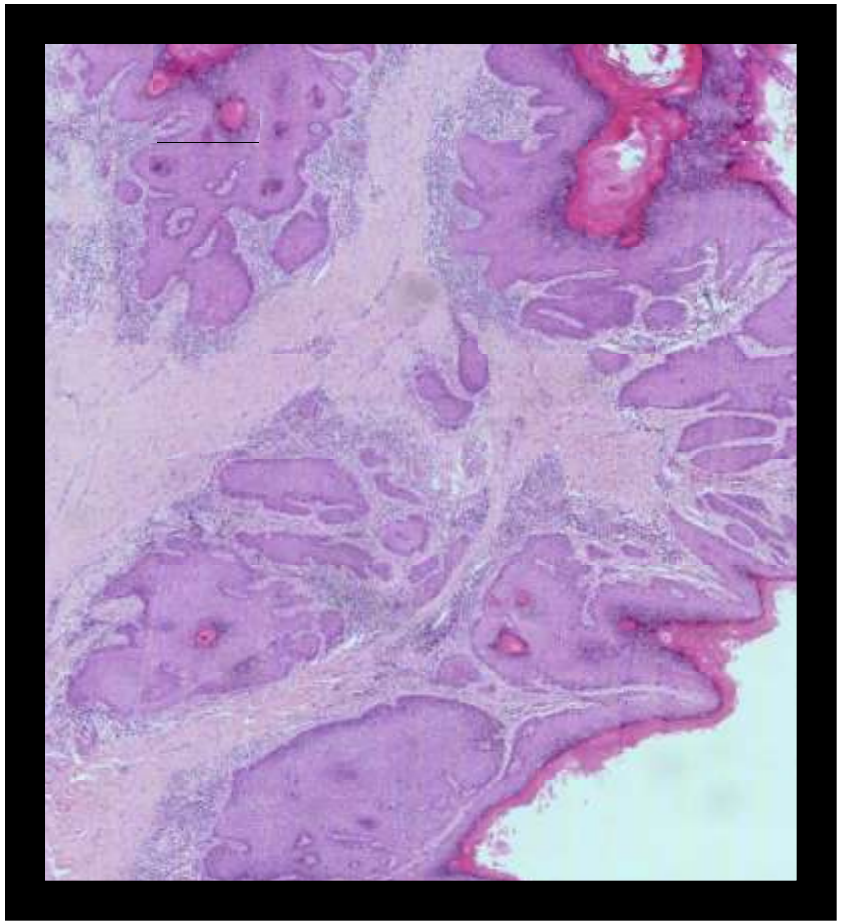
TDI sensor (CCD)

Tile scan

Standard microscope + CCD camera + Robotic stage



Transferred image

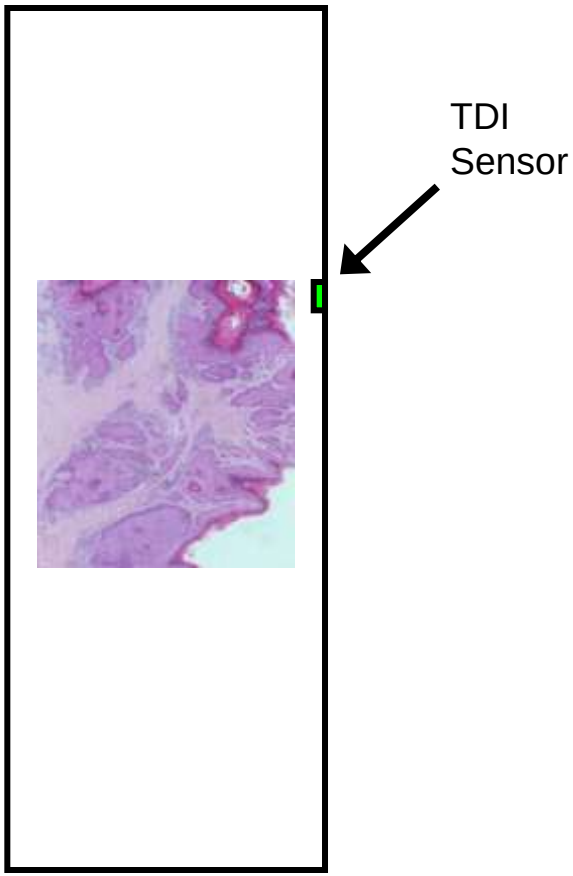


Line scan

Optics +TDI sensor + Robotic stage

Scanning

Slide



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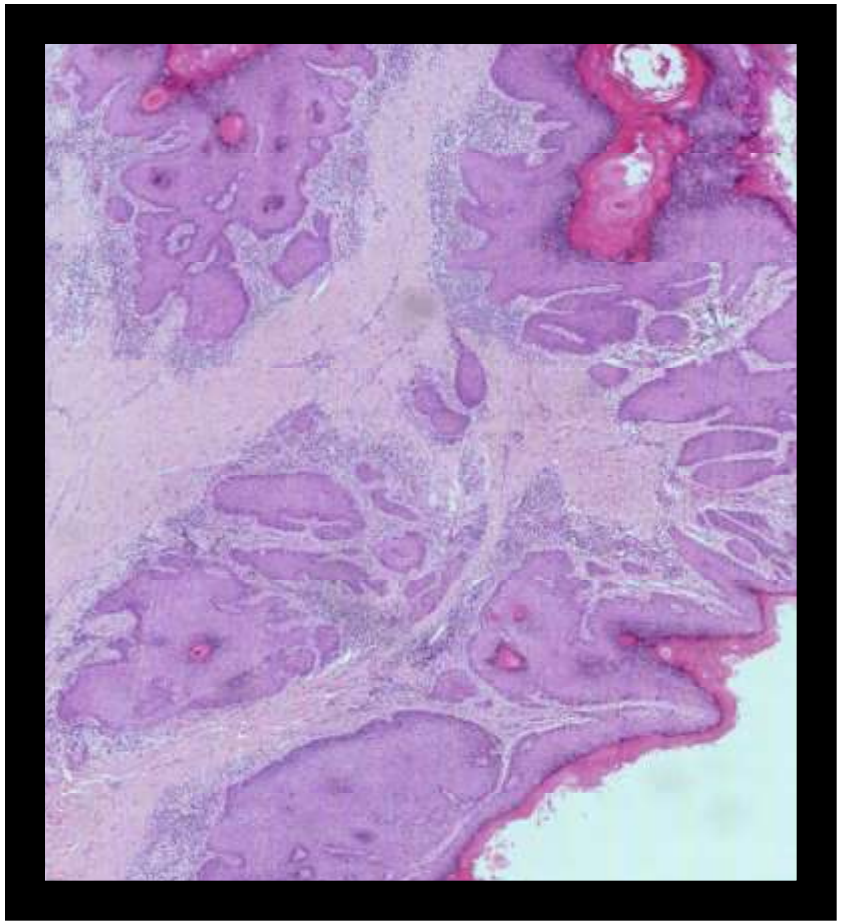
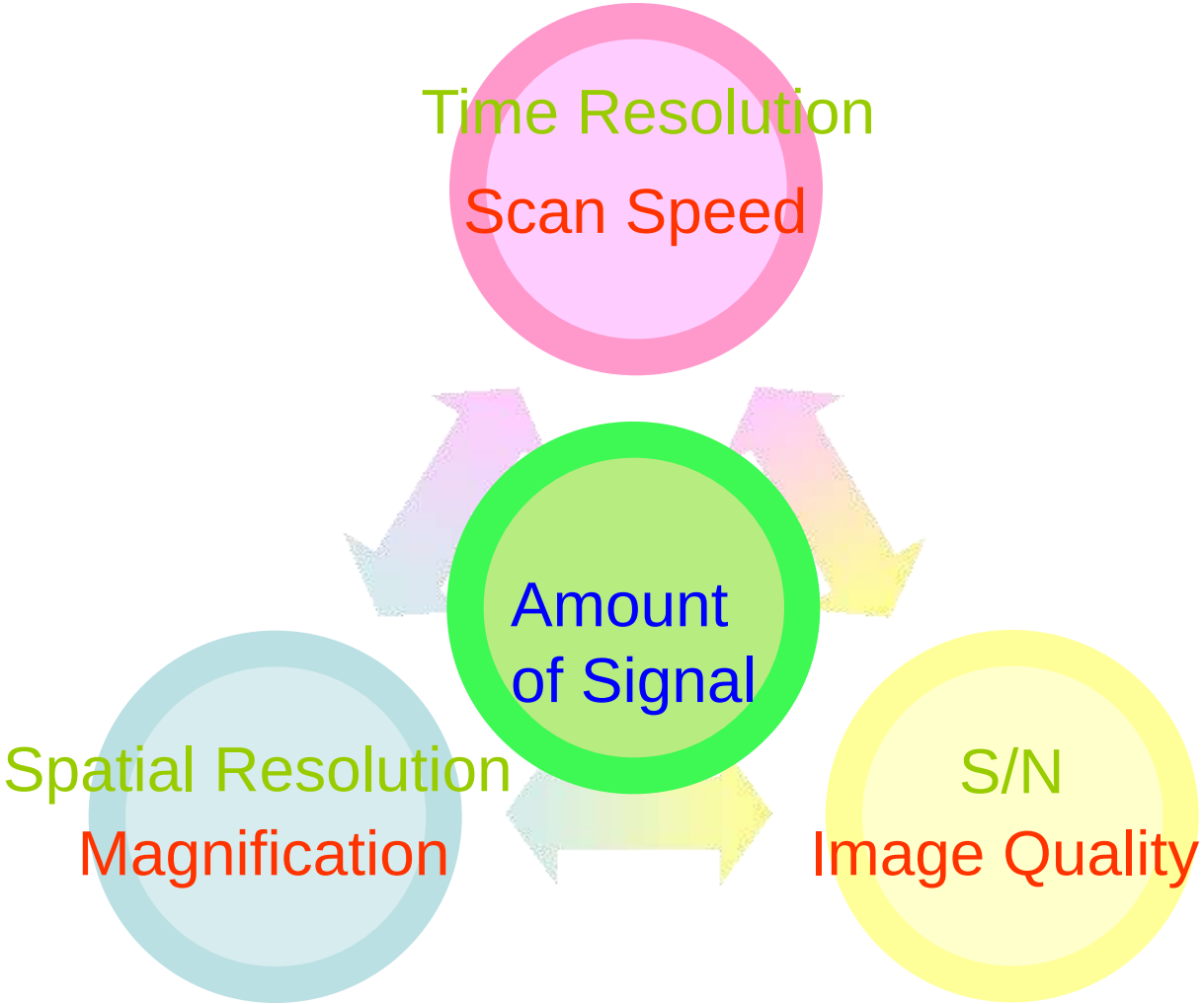


Eternal Triangle in microscopic imaging



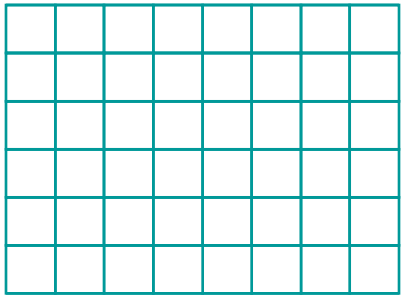
TDI sensor for fluorescence slides

TDI: Time Delay Integration

Motion of slide

readout signal

Line sensor



TDI sensor

Practical issues of fluorescence tissue slides

- Tissue recognition
  - Macro dark field illumination to make contrast for transparent tissues
- Focusing
  - Bright field focusing
  - Fluorescence focusing
- Auto fluorescence
  - Selection of fluorescence labels and fluorescence filter sets

Fluorescence

- Add-on option to standard NanoZoomers

Fluorescence

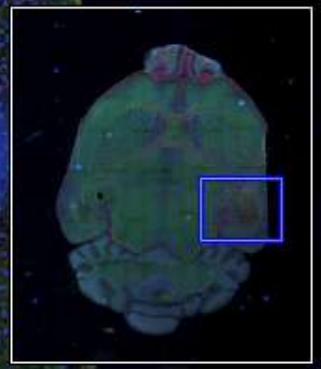
NDP
NanoZoomer Digital Pathology

Mouse Brain

Alexa Fluor 488 NF-H

Alexa Fluor 568 GFAP

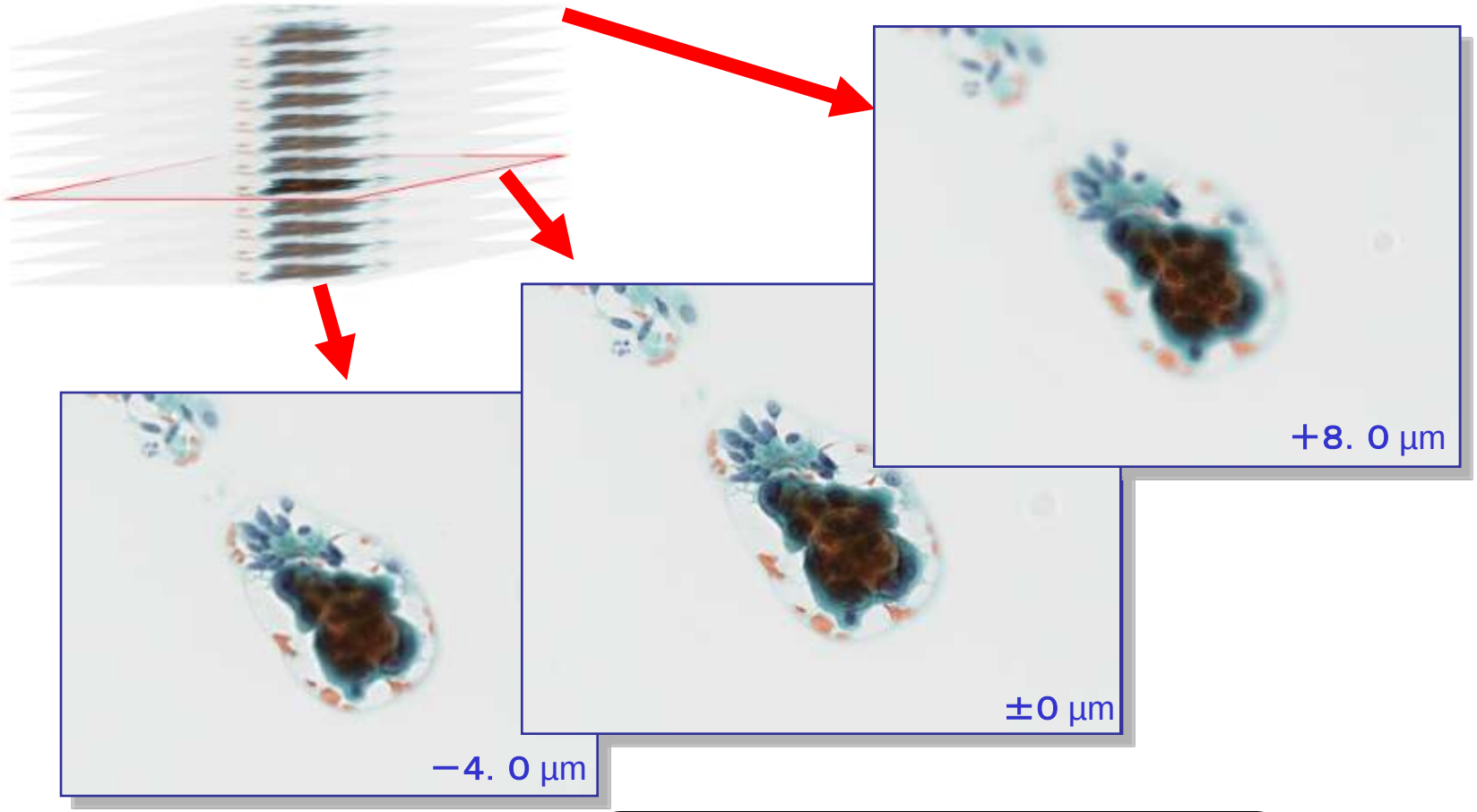
Pacific Blue Histone



Z stack scanning

NDP
NanoZoomer Digital Pathology

SELECT NUMBER OF PLANES AND SPACING FOR SCANNING

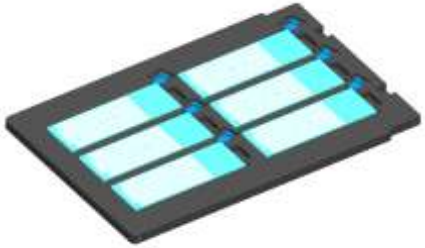


NAVIGATE THROUGH THE Z STACK WITH NDP VIEWER

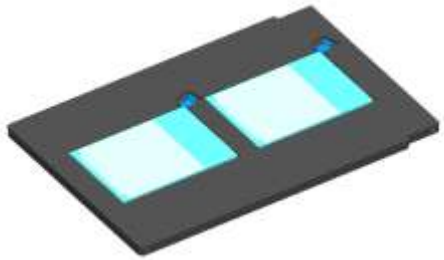
New NanoZoomer RS

- Compact and affordable

6 Standard slides



2 Double size slides



NanoZoomer Family

Type	NanoZoomer-HT	NanoZoomer-RS
		
Sensor	3 Color TDI CCD	
Slide Loader	210 Slides Cassette Unit	6 Slides-Standard 2 Slides-Double(Option) Slide Tray
Scanning Speed 20x20mm/20x	3 Minutes	
Fluorescence	Option	
Size (W)	850mm	550mm

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