

How to make Cytopathology Case Videos, CCVs

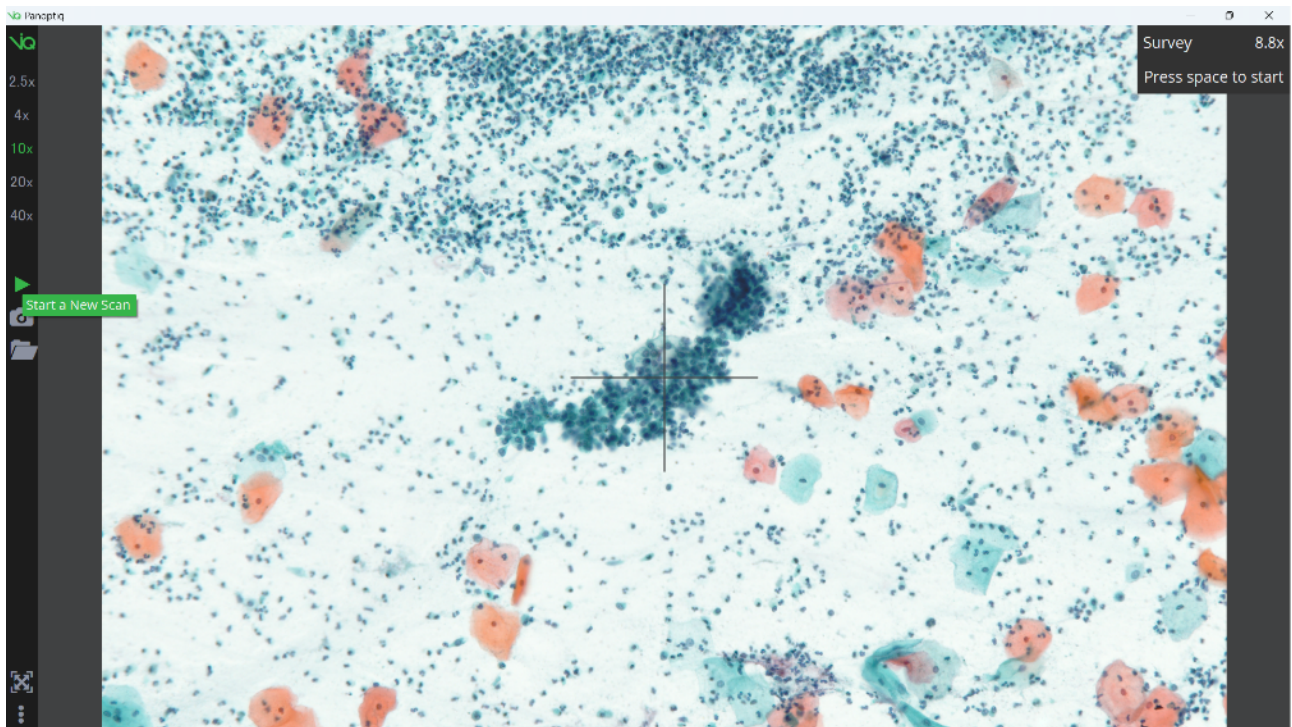
Cytopathology Case Videos, CCVs, are 2-4 minute-long Internet videos that reproduce the process of observing cytological slides under a microscope with changing focus. Presented is a detailed description of how we typically create CCVs.

I: What to prepare.

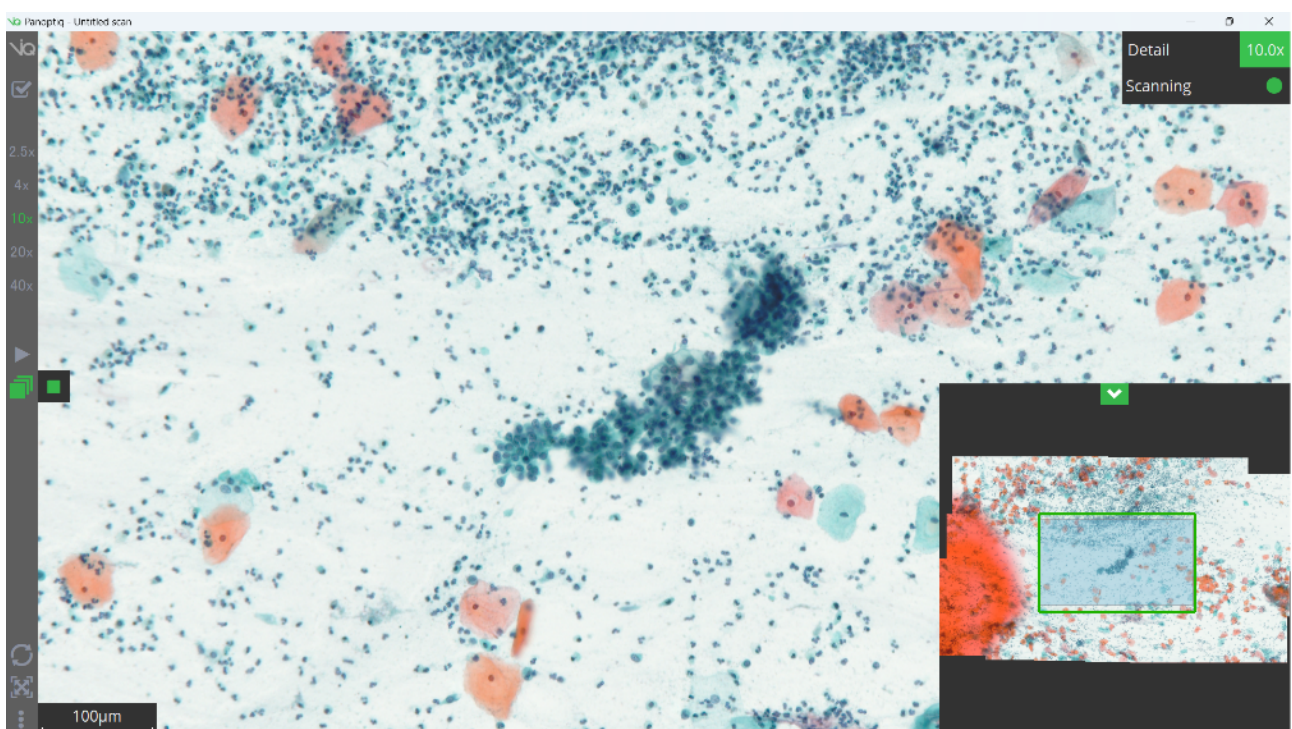
- 1) Cytology glass slides that were marked by a cytologist.
- 2) Microscope: Nikon, ECLIPSE Ni-U, with 10x and 40x objectives.
- 3) Digital camera: Point Grey Research Inc, BFLY U3 23S6C-C, 1920-1200 pixels.
- 4) Computer A: Dell Laptop computer with Windows 11 installed, a USB type-C port and Panoptiq (ViewsIQ) installed.
- 5) Computer B: MacBook Air, 16-inch with Mac OS 15 or 26 and the Keynote application and OmniConverter(Omni) installed.
- 6) External Storage Device: USB type-C, SSD, 1TB.

II: Use Computer A to create a panoramic image of the microscopic field and record the microscopic field with changing focus.

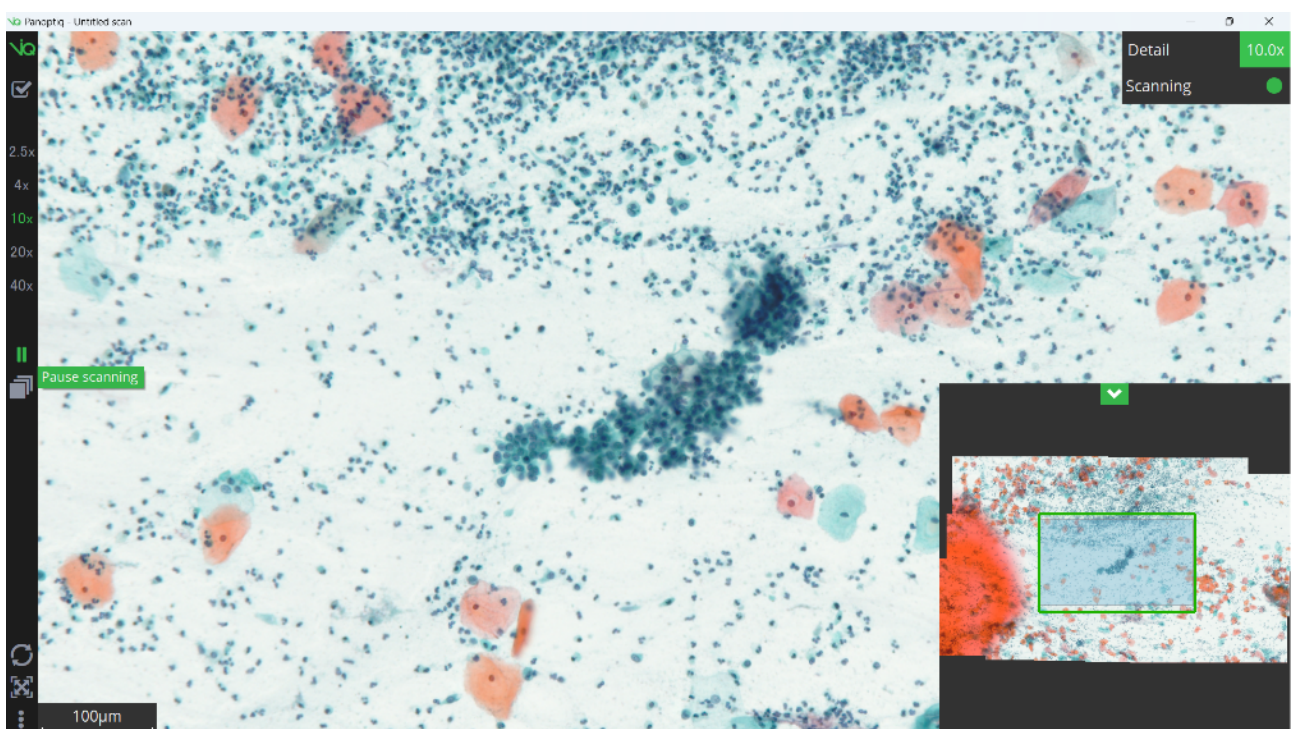
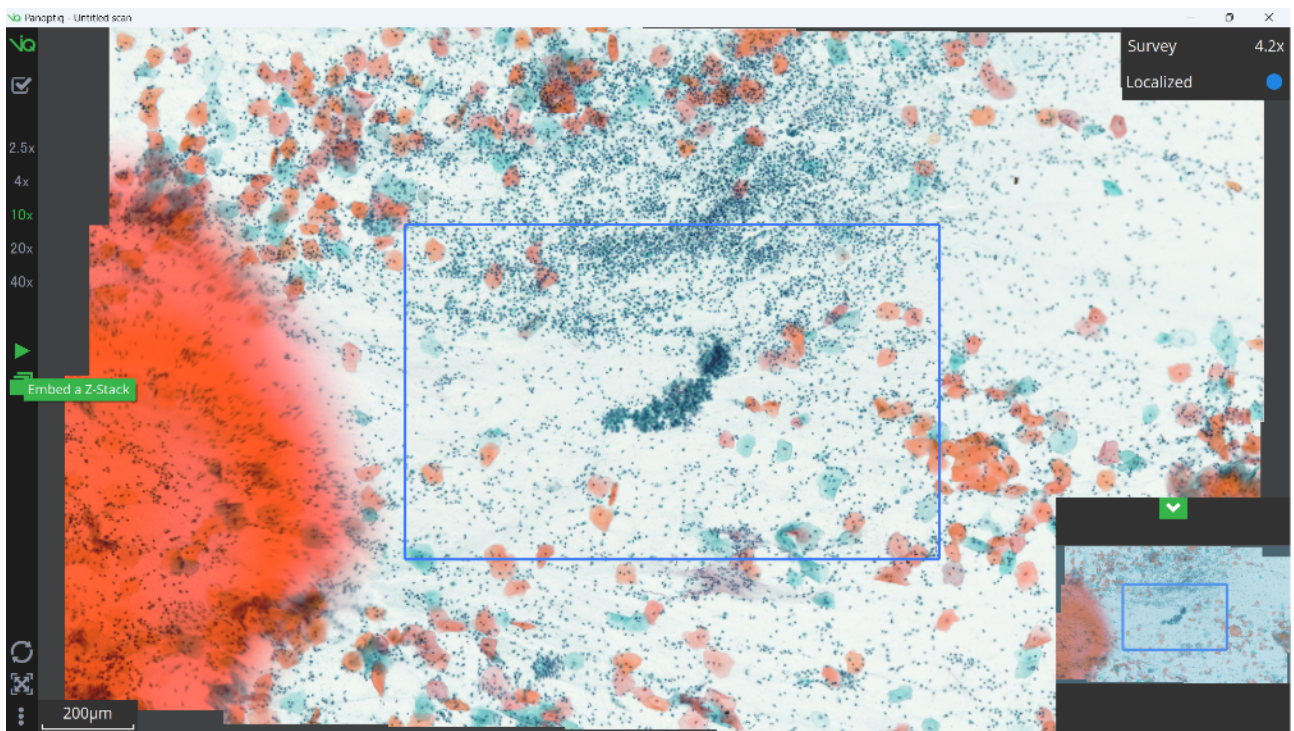
- 1) Launch Panoptiq. ViewsIQ recommends Panoptiq 5, but we use Panoptiq 4.
- 2) Calibrate the White Balance. It is recommended to calibrate the white balance for each glass slide. Do this for both the 10x and 40x objectives.
- 3) Creation of Panoramic Images. Panoptiq can only capture videos in areas that have been created as panoramic images. To create a panoramic image, use the 10x objective and select the corresponding button on the application screen. To create a panoramic image, it is necessary to always focus the microscope on the nucleus of small lymphocytes and polynuclear white blood cells in the deepest area. It is essential not to try to focus on the nuclei of tumor cells. It is not possible to create panoramic images without an object to focus on. Therefore, even if an LBC slide is used, it will be very difficult if the number of cells is extremely low. You may have to abandon creating a panoramic image of the whole slide. With conventional slides, panoramic images can only be created for limited areas. (Fig. 1)



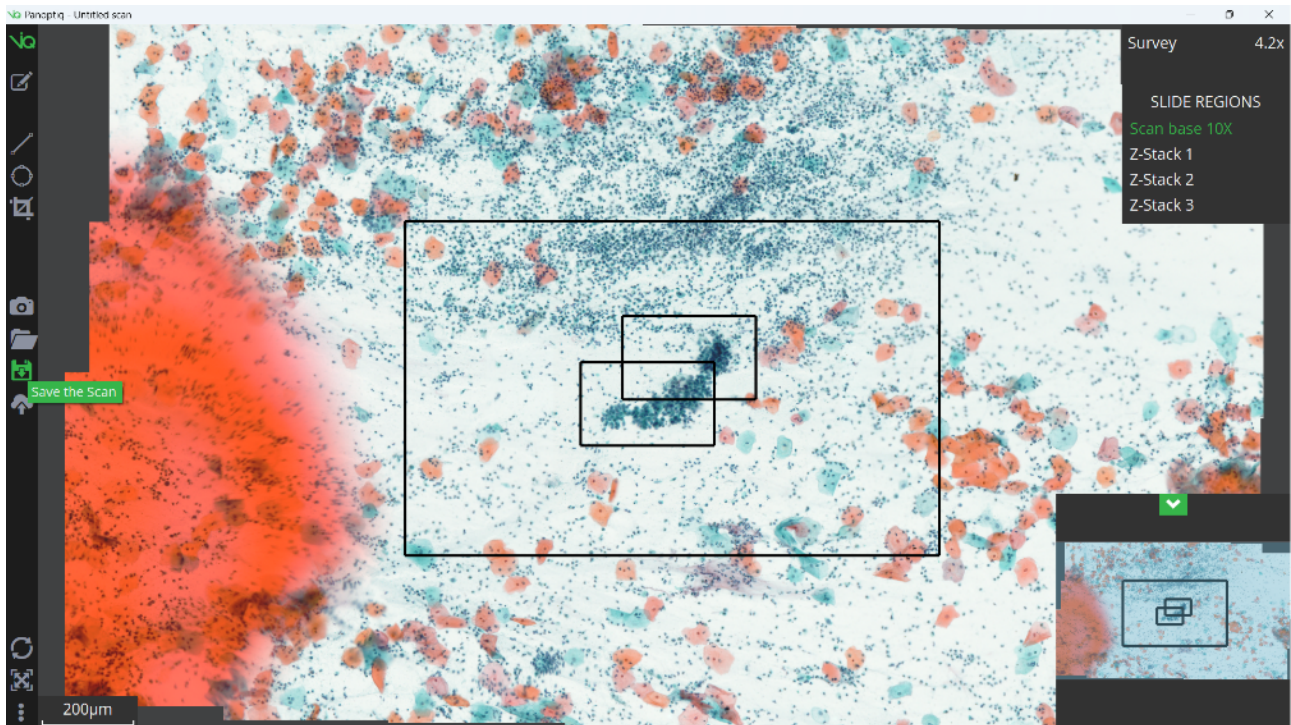
- 4) Determine the microscope field for video recording using the 10x objective. Panoptiq can only record video in the areas where a panoramic image was captured. Press the Start button from the video shooting mode, then use the fine-adjustment knob to move the microscope stage as uniformly as possible over 2–3 seconds, shifting the focus from the top to the bottom of the cell or cell group. After repeating the process of changing the focus 2-3 times, stop the video recording. The recorded area will be displayed in a black frame. In Panoptiq, a microscopic image with changing focus is called a Z-Stack image (Fig. 2)



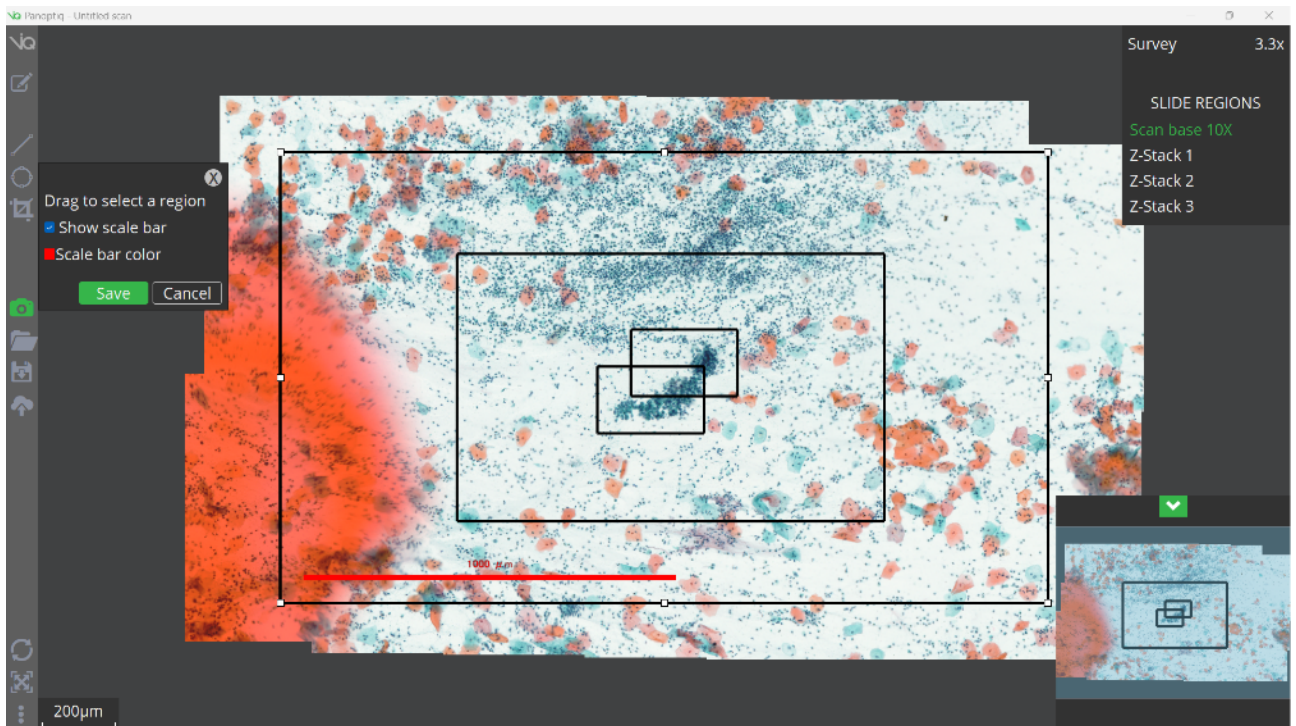
- 5) Next, determine the microscopic fields for video recording with the 40x objective. The number of microscope fields of view is usually 1-3 per area recorded with the 10x objective. The video recording time is about 4-5 seconds, longer than when using the 10x objective. Repeat this operation 3-5 times. If there are multiple fields of view with the 40x objective, repeat the video recording in the same way. The recorded areas are displayed in a black frame. (Fig. 3, 4)



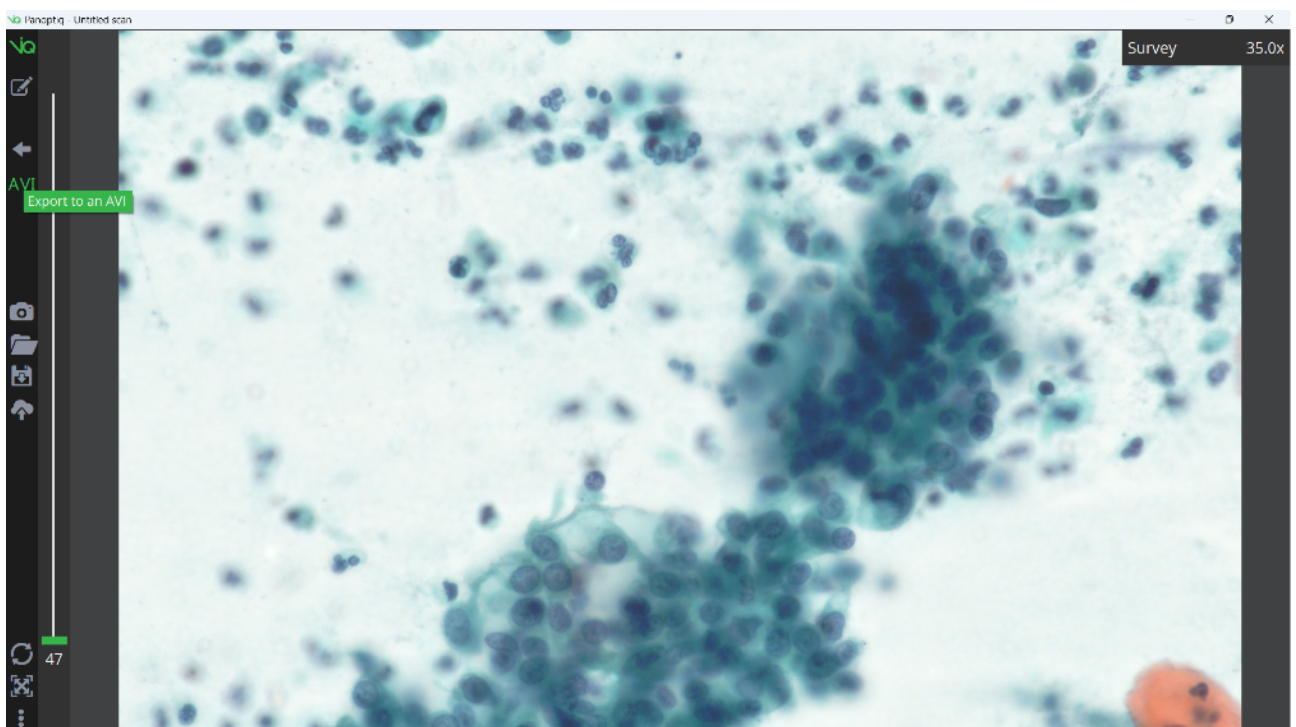
- 6) Repeat process 4) and 5).
- 7) When all video recordings are finished, save them. Save not only video recording data but also the panoramic images. Specify the external SSD as the save location. (Fig. 5)



- 8) Export the Panoramic Image. Include a scale in the panoramic image. If the subject is an LBC slide, first export the panoramic image of the whole slide. Next, export the panoramic images containing the areas that were video recorded. The number may be up to 7-8 images. Specify the SSD for storage. Save as a JPEG file. For a conventional slide, specify the range of the panoramic images containing all video recorded areas and export it. The recorded areas are often separated, in that case, it may be necessary to create several panoramic images. (Fig. 6)



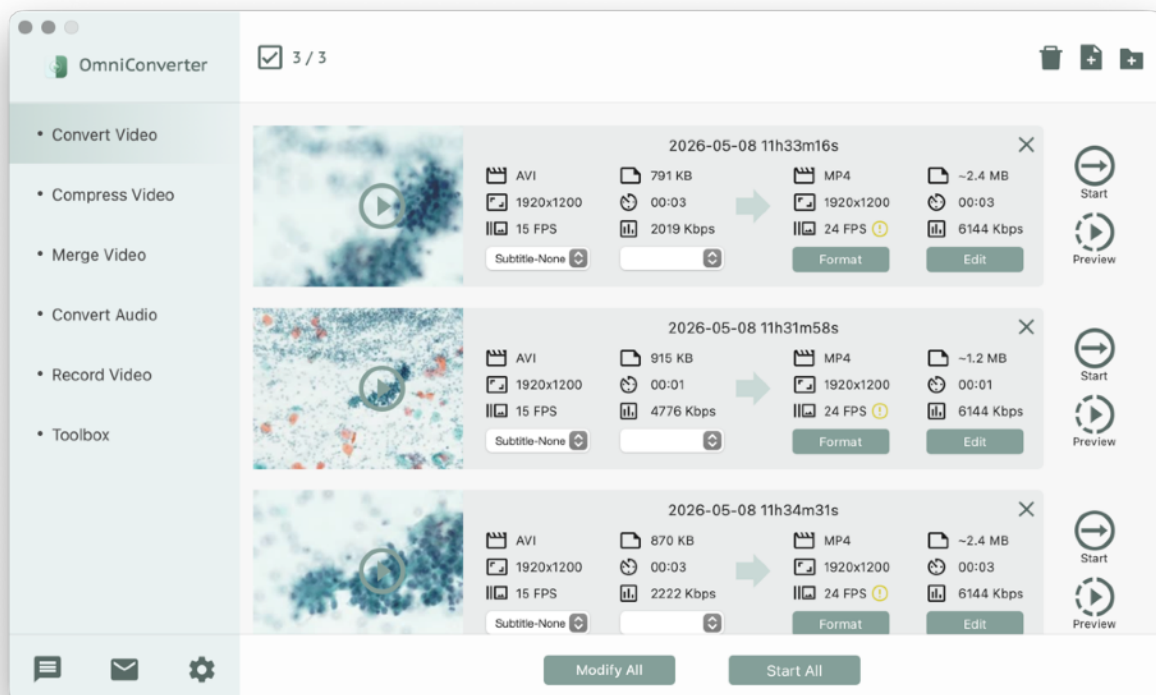
- 9) Use the slider of the individual recorded video screen to select the appropriate section from the start point to the end point and export it. The video is exported as a 15 frames per second AVI file, and the length will be adjusted to about 23 frames when recorded by the 10x objective. The length of the recorded video by the 40x objective should be about 47 frames. Specify the external SSD for storage. Make sure that all videos are exported as AVI files. In the end, there should be around 10-20 AVI files. (Fig. 7)



10) Exit Panoptiq and disconnect the SSD.

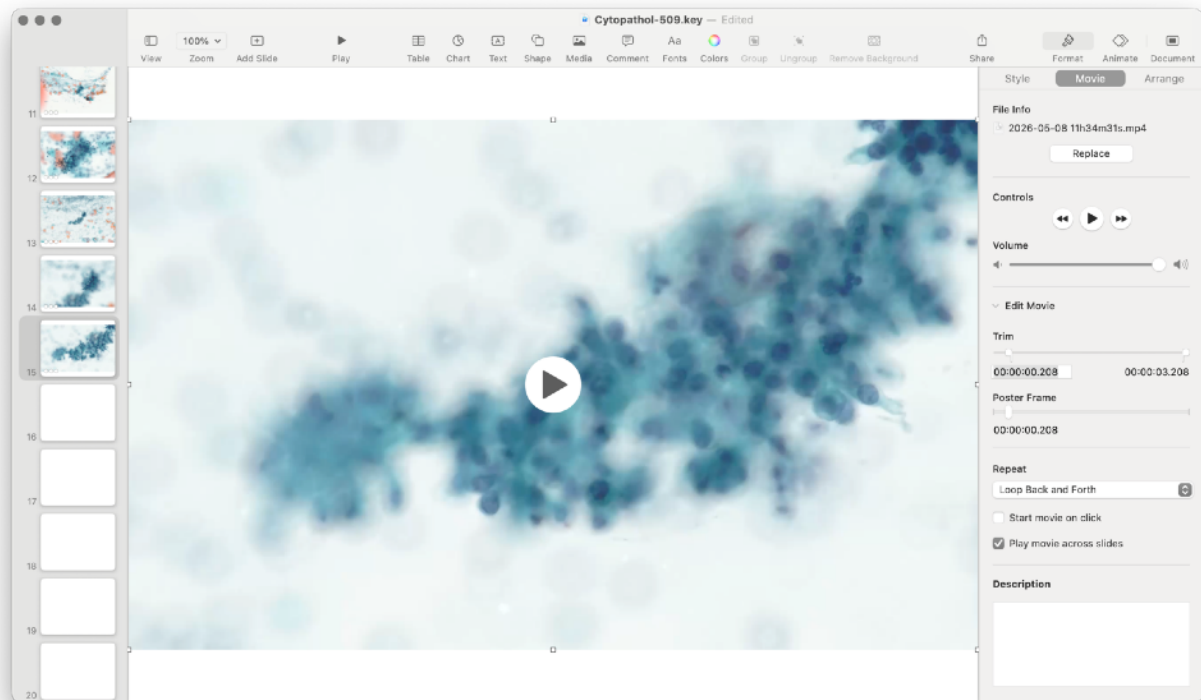
III. Paste the panoramic images and AVI files created with Computer A into the presentation file and export them as automatic playback video files. This will be performed on Computer B.

- 1) Connect the SSD and combine the panoramic images and AVI files into a folder named after the slide. Apply a slight sharpening effect to the panoramic images using the Preview application. Open the AVI files with the OmniConverter application and convert them to MP4 files. These MP4 files are called Zavic, Z-axis video for cytology. Zavic is a name we previously proposed. (Fig. 8)

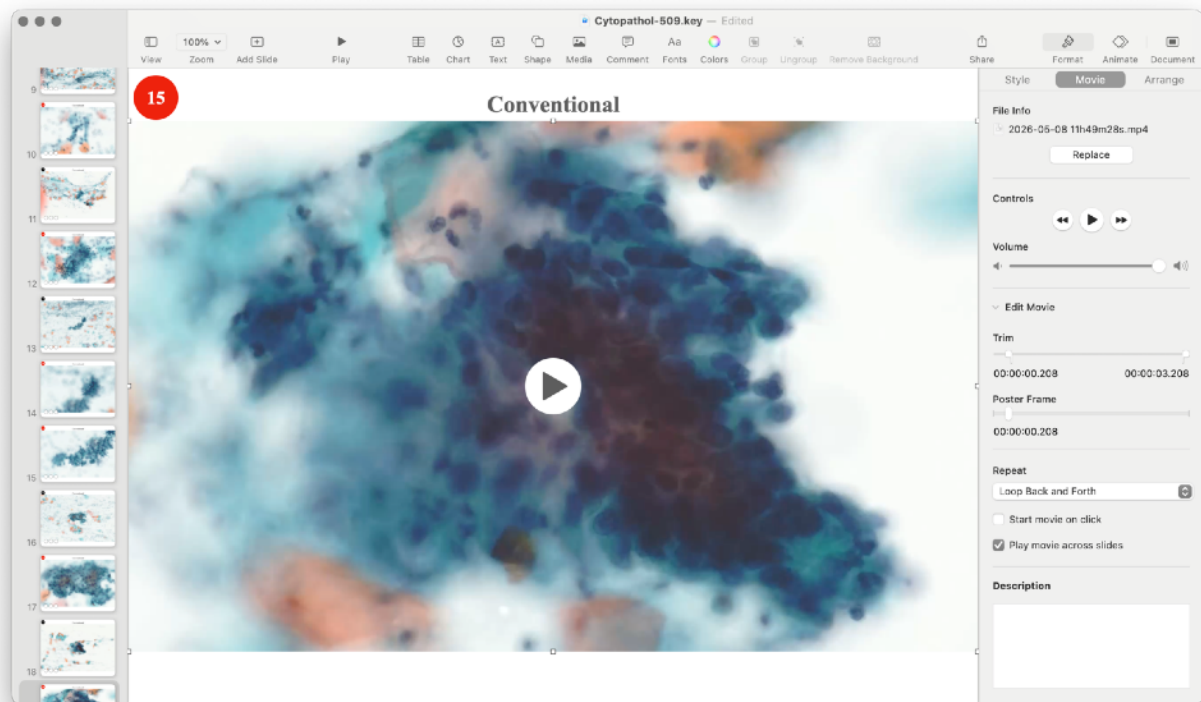


- 2) Launch the Keynote application and create a new file. Set the page aspect ratio to 4:3. On the first page, include the case introduction. Include the case number, approximate patient age and gender, specimen collection site, collection method, cytology slide preparation method, and the name of the facility that submitted the case. The name of the facility that submitted the case can be kept confidential.
- 3) Paste a panoramic image on the second page. For LBC slides, paste JPEG files that have been cropped to include the video recorded areas on the next page. When pasting multiple images, each image has a scale, so adjust the size according to the image scale. For conventional glass slides, the overall panoramic image will be omitted.
- 4) Starting on the next page, paste the Zavics one by one in the center of each page in the order they were recorded. Edit the Zavics. Set it to repeat playback and reverse playback, and trim the file length so that the recording with the 10x objective is about 1.4 seconds, and the recording with the 40x objective is

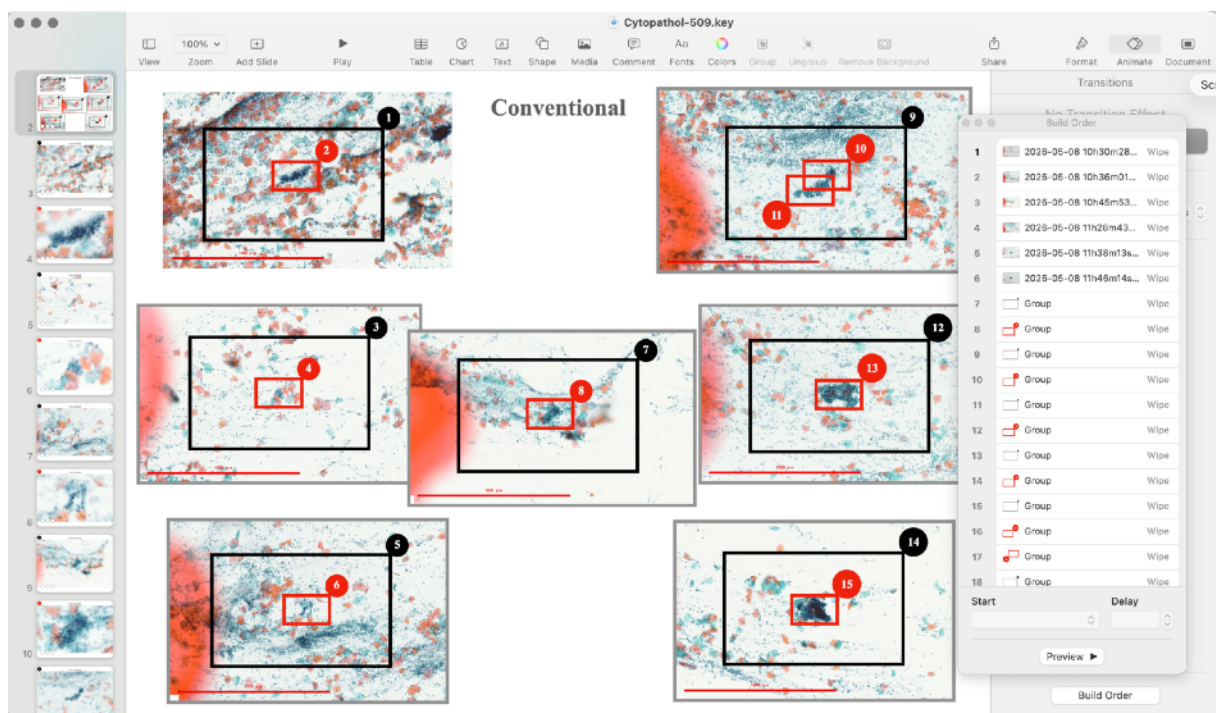
about 3 seconds. Uncheck "Start by clicking". The Keynote application makes it easy to set up repeated playback & reverse playback. (Fig. 9)



- 5) Perform process 4) for all attached Zavics. The total number of Zavics will be about 10-20 per sample. In some cases, the glass slides provided may have been prepared using two methods: conventional glass slides and LBC slides. Even in that case, the recommended number of Zavics is around 20. Be aware that if the number of Zavics exceeds 20, the playback time of the completed CCV may exceed 5 minutes.
- 6) On the page where the Zavic is pasted, a description of the glass slide preparation method (conventional method or LBC method) will be added, along with a circled number in the upper left corner. A 10x objective is represented by a black circle, and a 40x objective by a red circle. (Fig. 10)

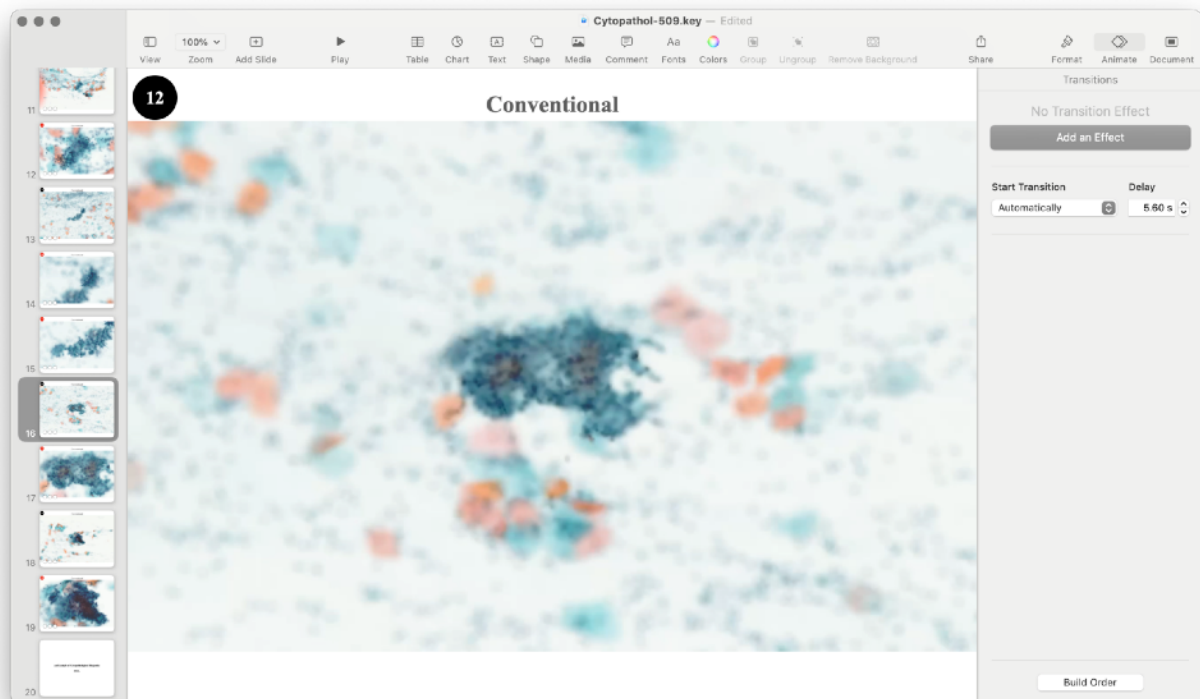


- 7) Add the video-recorded areas as numbered rectangular frames to the page created in step 3) above. These will appear sequentially via a 'wipe' transition. The 10x objective is displayed with a black frame and a black circled number, while the 40x objective has a red frame and a red circled number. (Fig. 11)

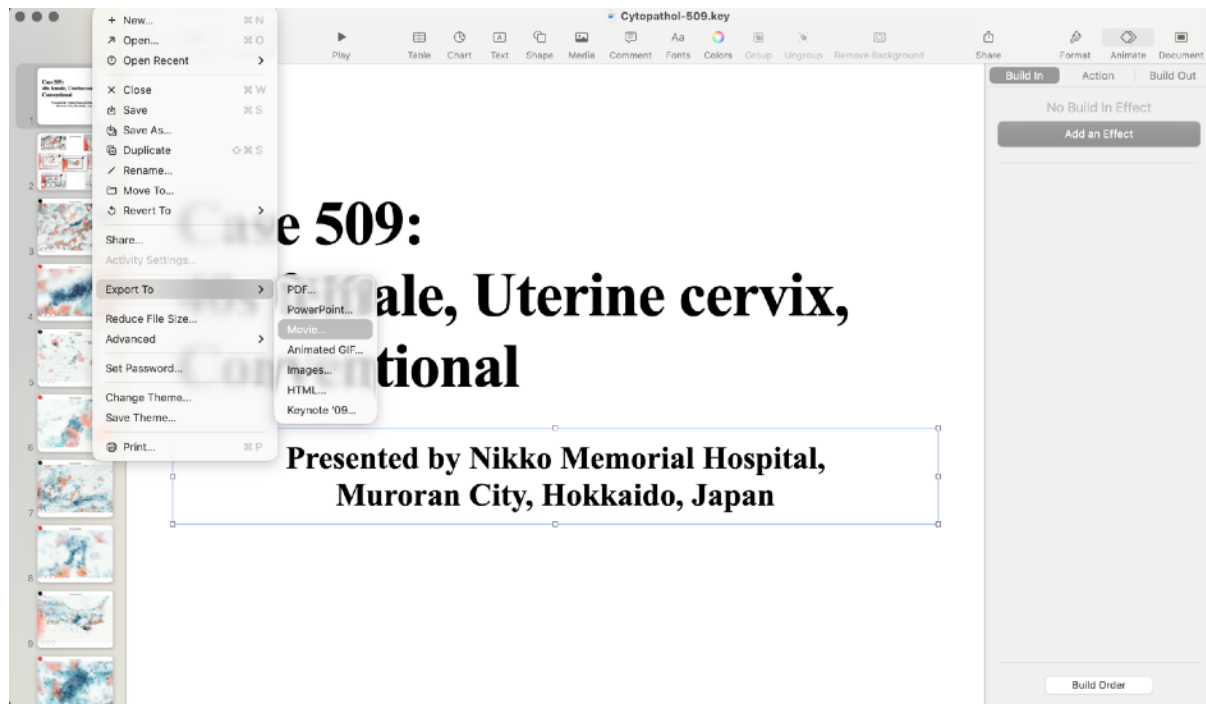


- 8) The last page describes an example of cytopathological diagnoses.

- 9) The Keynote file is set to play automatically. The page transition and duration of display are determined and set for each page. The first page is displayed for 5 seconds and the next panoramic image is displayed for 2 seconds. After that, the panoramic jpeg files are pasted sequentially, and the numbered rectangle frames are displayed in order, it takes 2 seconds for page transition. Pages containing a Zavic are displayed for 5.6 seconds for 10x objectives and 12 seconds for 40x objectives. Each Zavic will repeat forward and reverse playback two times. The last page, containing the cytopathological diagnosis, is displayed for 5 seconds. (Fig.12)



- 10) Export the Keynote file set to playback automatically as a video M4V file. Change the file extension to .mp4 manually. /File/Export To/Movie... (Fig. 13)



IV. Log in to the Vimeo site and upload the mp4 files created on Computer B.

- 1) Upload the MP4 file created on Computer B.
- 2) Set up file publishing and set up thumbnails.

V. Manage information about the created CCVs.

- 1) Manage using the Numbers application on Computer B.
- 2) Management items include: the case management number, Vimeo URL, CCV length (seconds), number of Zavics, name of facility providing case, case number assigned at providing facility, date of specimen submission at providing facility, clinical department, date of initial upload to Vimeo, specimen type, specific specimen details, presence of conventional slides, presence of LBC slides, presence of cell block method specimens, patient sex and approximate age, and case summary.
- 3) Export case information for the CCVs portal site as an XLSX file and provide it to the portal site administrator. Only the case management number, Vimeo URL, date of initial upload to Vimeo, specimen type, and specific specimen details should be provided.

Some parts of this guide might be difficult to understand, but please feel free to contact us for assistance. It may also be helpful to consult with someone close by who is familiar with Windows 11 or Mac OS.

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