# ACCU-*Slide*MS Manual

Software-Version 2019c (2019-09-25)

Digital Slide Scanning Simplified

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# Compatibility

## Intended Use – Is manual scanning suitable for your scanning task?

Manual slide scanning is a good solution for many situations. However, there are several situations where manual slide scanning is not feasible. ACCU-SCOPE can assist you in determining if manual slide scanning is a good choice for your work.

For comparison, a few reasons for manual and automated scanning solutions are listed below. Most of the discussion relates to the time required to manually scan a slide. For example, where scanning biopsies at 20x takes only 2-3 minutes for a tissue section, scanning a large sample (>20x20mm²) at 40x can easily take an hour or more.

### Reasons to consider manual slide scanning:

- your samples should be scanned at low magnifications (2x, 4x, 10x)
- your samples need to be scanned at 20x or 40x magnification, and the specimens are small or it is acceptable to scan a subregion (up to 10x10mm²)
- your samples have a clear outline and all parts of the tissue on the slide are connected
- you only need to scan slides occasionally

### Reasons to consider motorized slide-scanning:

- you need to scan many slides on a regular basis (volume)
- the barcode labels of your slides should be read automatically
- the sample sizes on your slides are large (> 20x20mm²) and need to be scanned at 40x (high) magnifications
- your samples contain multiple areas that are not connected to each other

# Compatible Microscopes

In general, most microscopes are compatible or can be upgraded to meet the requirements for manual slide scanning. The most critical part is the illumination (See chapter Illumination).

## Why is the Microscope Illumination Critical for Manual Scanning?

Acquiring images while moving the stage requires very short camera exposure times to avoid motion blur. Exposure times need to be at or below 1 millisecond (ms) to ensure sharp images. To reach these short exposure times, it is necessary to direct as much light as possible to the camera.

## Good Combinations for Scanning

While many combinations of objective lens magnification, microscope coupler magnification and camera are possible, not all of these combinations are efficient.

The goal is to select a combination that can resolve the desired details, but avoids the so-called empty magnification (Structures appear larger, but do not resolve additional details).

### Explanation of "Empty Magnification":

Imagine you have a picture 1000x1000 pixels. You open the picture in image processing software and resize it to 5000x5000 pixels. Are the edges any crisper or sharper? No. But you did succeed in increasing the file size by 2500%.

Assume a 40x/N.A. 0.65 objective lens sends an image of 1200x1200 "pixels" to a camera, where 1 pixel is the size of the smallest “resolvable” detail. That means that a camera with a resolution of 1200x1200 pixels can resolve every detail present in the image. Acquiring the image with a higher resolution camera does not add any real information, but it does add size to the image file AND the image looks blurry. This is called empty magnification.

Disadvantages of empty magnification for manual slide scanning:

1. The more an image is magnified, the smaller the field of view of the camera gets (e.g. empty magnification of 2 results in 4 times the scan time)
2. Empty magnification demands more light from the light source (2x empty magnification => 4x light necessary)
3. Resulting size of the scans get bigger (2x empty magnification => 4x file size)

Sample combinations of objective lens, camera and sensor sizes and the resulting performance:

| Objective lens (mag / N.A.) | camera adapter magnification | Image sensor: 1/1.2", 1936x1216 | Estimated scan times 10x10mm | Image sensor: 1/1.8", 2048x1536 | Estimated scan times 10x10mm | Image sensor: 2/3", 2448x2048 | Estimated scan times 10x10mm |
|---|---|---|---|---|---|---|---|
| 10x / 0.25 | 1x | very good | 3 min | slow | 5 min | slow | 3 min |
| 10x / 0.25 | 0.65x | critical | 2 min | very good | 3 min | very good | 2 min |
| 10x / 0.25 | 0.50x | not resolved | 2 min | excellent | 2 min | excellent | 2 min |
| 20x / 0.40 | 1x | good | 7 min | very slow | 13 min | very slow | 9 min |
| 20x / 0.40 | 0.65x | excellent | 4 min | good | 6 min | good | 5 min |
| 20x / 0.40 | 0.50x | critical | 4 min | very good | 4 min | very good | 4 min |
| 40x / 0.65 | 1x | slow | 23 min | very slow | 45 min | very slow | 29 min |
| 40x / 0.65 | 0.65x | excellent | 12 min | slow | 20 min | good | 13 min |
| 40x / 0.65 | 0.50x | critical | 10 min | good | 13 min | good | 10 min |

Image quality relative to the optimum megapixels of a scan:

not resolved = < 70%
loss of detail = 70 - 90%
excellent = 90 - 125%
very good = 125 - 175%
good = 175 - 250%
slow = 250 - 400%
very slow = > 400%

critical => the image sensor is too large for the camera adapter. This combination will not work for slide scanning because the corners of the camera image will be dark (vignette). For a single still image, however, the image of the sensor can be cropped to remove the dark corners.

## Cameras

Currently, these camera vendors and cameras listed below are supported. Please contact ACCU-SCOPE for compatibility with another camera that you may already own.

**Basler**

- Basler Ace 2.3MP and Ace 3.0MP
  - acA1920-40uc
  - acA2040-55uc

**Recommended camera depending on camera adapter*:**

| Camera Adapter | Basler industrial cameras |
|---|---|
| 0.5x | acA2040-55uc (3.0MP) |
| 0.63x / 0.65x / 0.70x | acA2040-55uc (3.0MP) |
| 1.0x | acA1920-40uc (2.3MP) |

* These are general recommendations based on camera sensor size and data readout rates. For specific scanning tasks, other combinations may produce better results or reduce scanning time significantly.

## Compatible Viewer Software

ACCU-*Slide*MS software creates a pyramidal tif file (or SVS file) and is not shipped with a software for viewing, annotating or analyzing the scan.

The scanned file is known to be readable by the Viewers/Libraries listed below:

| Name | Company | URL | Description |
|---|---|---|---|
| Sedeen | Pathcore | https://pathcore.ca/sedeen/ | Versatile stand-alone viewer. Royalty free for research. |
| easyzoom | SmartInMedia GmbH | https://easyzoom.com | Easy to use web platform to share slides (2GB free storage) |
| Zoom | Microdimensions GmbH | https://micro-dimensions.com/zoom/ | Fast, free viewer |
| Pathology Cloud | Proscia | https://proscia.com/ | Easy to use web platform to share slides. Currently 50GB of free storage. |
| ViewPoint | PreciPoint GmbH | http://www.precipoint.de/microscopy-software/viewpoint/ | Free open slide-based viewer. Excellent Multi-touch Support |
| OpenSlide | | http://openslide.org/ | Open source library to read various whole slide image formats |

| | | | |
|---|---|---|---|
| Medical Image Manager | HeteroGenius | http://www.medicalimagemanager.com/static/MIM/index.html | Web-based Desktop viewer. Supports 3D Pathology, Multi Stain Analysis, Automatic Annotation, Tissue Quantification. Basic version is free |
| PMA.start | Pathomation | http://free.pathomation.com/ | Webbased Desktopviewer |
| ... | ... | ... more viewers will be listed as soon as they are tested ... | |

Other viewers may be compatible for viewing ACCU-*Slide*MS files, but have not been evaluated for performance.

## Computer Hardware Requirements

Only fast computers with high-resolution displays are compatible with manual Whole Slide Imaging.

| **Hardware** | **Minimum Configuration** | **Recommended Configuration** |
|---|---|---|
| CPU | Intel Core i3 Dual Core 3000, 4000, 6000 series | Intel Core i5 or i7 Quad Core 4000/6000/7000 series |
| RAM | 4 GB | 16 GB |
| DISK | 500GB Standard Harddrive | 512GB Solid State Drive |
| Chipset | Intel 7 Series B75/Q77/H77/Z77/... | Intel 100 Series H170/Z170/... or Intel 200 Series |
| Display | Resolution 1600 x 900 pixels | 23" Display / 1920x1080 pixels (good)<br>27" Display / 2560x1440 pixels (better)<br>32" Display / 3840x2160 pixels (best) |
| Operating System | Win7 Home 64bit SP1 | Windows 10 Professional 64-bit |

ACCU-*Slide* software is x64 and will only work on 64-bit operating systems.

**CAUTION:** Portable computers (laptops) are not recommended!

**ADDITIONAL NOTES:**

- The processor MUST be from Intel and MUST be at least the 3000 series (e.g. Intel i7 3770). This series and newer are USB 3.0 chips, important for camera performance.
- If you must use a portable computer, ensure it is equipped with an Intel Quad-Core processor (e.g. i7 6700HQ or i7 7700HQ) and minimum 8GB RAM.
- Older Quad-Core i5 and i7 computers will also work, but MUST be upgraded with a USB 3.0 add-in-card with NEC/Renesas host controller or Fresco Logic FL1100 host controller (e.g. Delock 89391 or Exsys EX-11081-2)

# Setting Up the Scanning System

## Mounting Camera

1. Avoid dust in the optical system!
2. If the trinocular head of the microscope has a light path selector, set it to send 100% of the light to the eyepieces.
    a. If less light goes to the camera, ensure that the eyepieces are covered to avoid straylight from entering the optical path.
3. If a camera is currently installed on the microscope, remove the camera adapter with attached camera from the microscope.
4. Remove the old camera from the camera adapter.
5. Hold the new camera in a way that the sensor is always facing downward. This minimizes the possibility of dust falling onto the camera sensor.
6. While the new camera sensor is facing downward, remove the dust cap from the new camera. You may wish to mount the cap onto the old camera.
7. Attach the camera adapter to new camera. Keep the camera sensor facing downward.
8. Attach the camera and adapter assembly to the microscope.
9. While viewing a live image from the camera on a monitor, adjust the rotation of the camera/adapter assembly to ensure the X motion of the stage is exactly horizontal movement of the image on the monitor.

NOTE: Some cameras require 180° rotation to orient the image on the monitor to reflect the motion as seen through the eyepieces.

## Installing the Software

Preinstallation tasks:

1. Ensure that your computer meets the minimum requirements. Refer to Page 8 of this manual.
2. Install a compatible Viewer software (refer to Page 7). ACCU-*Slide* software is shipped without Viewer software.

Software Installation and Setup Instructions:

1. Install the driver software for your camera
    a. For **Basler** cameras install: http://info.accu-scope.com/docs/digital-imaging-assets/software/accu-slide/Basler_Software.exe (In the setup wizard, select “USB3” and “camera user”)
2. Download and run the ACCU-SlideMS setup file from http://info.accu-scope.com/docs/digital-imaging-assets/software/accu-slide/ACCU-SlideMS-setup.exe
    a. **Caution:** do not run the setup file directly from the browser (otherwise, the setup cannot continue after a required reboot)
    b. A Windows smartscreen warning may occur during setup: click on "more"->"run anyway" to continue the setup
    c. If an error occurs while downloading, click the retry button
3. After setup completion, the software will start automatically and ask to provide a valid license file.
4. To start the software, click the ACCU-*Slide*MS icon that was created on the desktop.

## Replace License Files

Sometimes, it is necessary to replace existing license files (e.g. to turn a demo camera into a fully licensed camera). The software itself only asks for replacing the license file when it has expired. As a result, the stored license file(s) must be deleted manually.

Perform these steps:

1. Press Windows Key + R Key simultaneously to start the "run" command line dialog box.
2. Enter this command in the text box (without the quotation marks):

    "%localappdata%\ACCU-SCOPE\ACCU-SlideMS\license"
3. A Windows Explorer window showing the license file folder will be displayed.

4. More than one license files may be in the folder. Delete all licenses (or move them to the desktop to have a backup) and add the new license file that you received with this software and camera.
5. Launch the software and check that the license file is accepted (the new license information is displayed in the title bar).

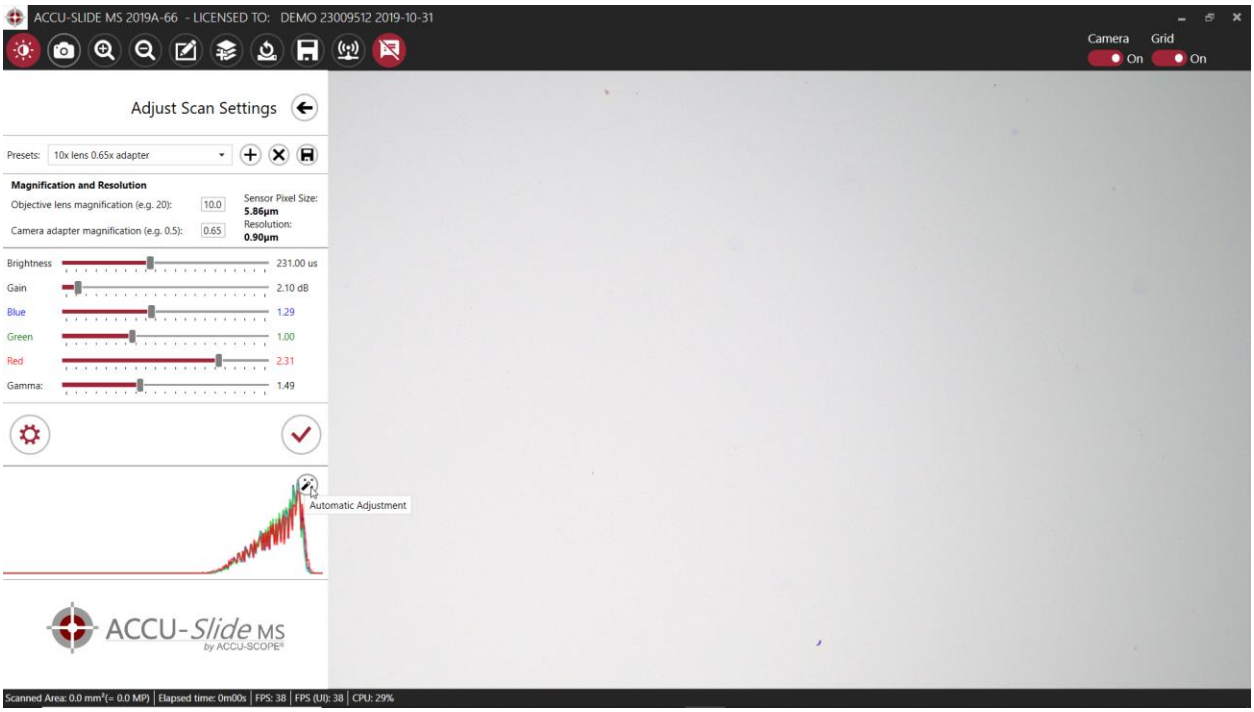
## Adjusting and Saving Scanning Parameters

**A) Adjust the scanning parameters:**

After launching the ACCU-*Slide*MS software and selecting the camera, the first step is to set up the scanning parameters.

1. Place a microscope slide on the stage.
2. Engage the desired objective lens for scanning.
3. If your microscope is using halogen illumination, be sure to use a light balancing filter (daylight filter). Additionally, the light source should have warmed up for at least 5 minutes to stabilize color and intensity of the illumination.
4. Locate and focus on the sample using the eyepieces.
5. Check if the sample can already be seen on the live camera preview (e.g. there is enough light).
6. If the live image appears black or very dark, ensure that all the light is directed to the camera vs. the eyepieces (e.g. 100%/0% 80%/20% 0%/100%). If it is, then increase the microscope illumination until the image is bright enough, or increase illumination to its maximum.
7. If the camera image is white, reduce Gain to 0dB and then reduce Brightness (= camera shutter time) until the sample can be seen on the camera.
8. Refocus the sample (focus of camera and focus of the eyepieces do not necessarily match).
9. Move to the edge of the sample until 90% of the camera image is empty/white background and 10% is sample.
    a. Make sure that the illumination is centered. Consult your microscope user manual for detailed instruction on centering illumination (a.k.a. Köhler alignment).
10. Adjust brightness and gain until the three peaks of the histograms can be seen completely without being cut off at the right edge.
11. Higher Gain and Brightness values will both increase the brightness of the image. Higher Gain values introduce more noise into the images. Higher Brightness values introduce more motion blur into the images. Try to keep Gain below 10dB and Brightness below 250µsec.
12. The brightness can be varied by adjusting the sub stage condenser's numerical aperture (e.g. condenser diaphragm), too.
13. Click the Automatic Adjustment button  in the upper right-hand corner of the histogram to automatically white balance the camera. You may also adjust the red and blue channel sliders separately until the red and blue peaks match the location of the green peak as close as possible.
14. Store the settings (see **section B** below).

Watch a video tutorial on adjusting scanning parameters.




**B) Establish and Save Presets**

In newer software versions, it is possible to save configurations for each objective lens magnification (and even for different stains).

1. Adjust the scanning parameters as described in **section A** above.
2. Press the "+" button to add a new preset. The dropdown list turns into a text box.
3. Enter a name for the setting (e.g. "10x NA0.25 HE").
4. Press the ENTER or RETURN key on the keyboard to save the setting

**C) Recall the presets**

1. Engage the desired objective lens in the light path. (e.g. 10x)
2. Select the corresponding preset from the list of stored settings.
3. Check that the brightness of the image is acceptable.
4. If the image does not look as expected, check that…
    a. the sub stage condenser aperture is set correctly (sufficiently open)
    b. the brightness of the microscope is set correctly
    c. the maximum amount of light is directed to the camera on trinocular heads
    d. confirm that the halogen lamp has been warmed up for a few minutes

**D) Update existing presets (in case microscope conditions have changed)**

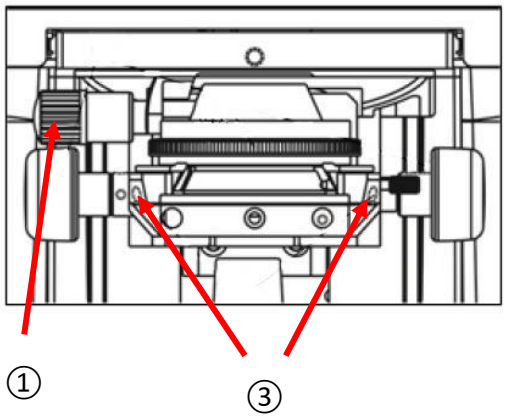
1. When halogen bulb is getting old or other scanning conditions changed, existing settings should be updated. Select the corresponding preset from the list of stored settings.
2. Select the desired preset.
3. Fine tune the settings.
4. Press the update button to update the existing preset setting.

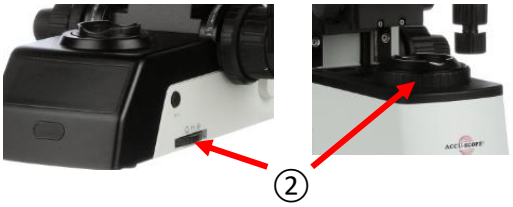
**E) Delete presets**

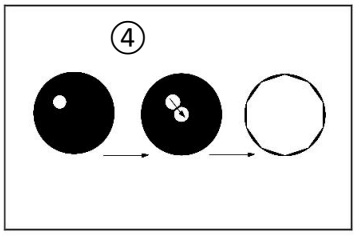
1. Select a preset that is not used anymore
2. Press “-“ button

## Aligning the Condenser

1. To avoid unevenly illuminated samples, it is important to carefully align for Köhler Illumination.
2. Detailed instructions are below, and videos can be found on YouTube.
3. Focus on the specimen using a 10x objective.
4. Using the condenser focus knob ①, rack/focus the condenser upwards so that it is either at the top of the its travel or is just below the slide.
5. Close the field diaphragm ②. You should see the leaves of the field iris diaphragm in the field of view ④. The field diaphragm (a.k.a. field stop) is typically located around the light well or below the light well in the base of the microscope.
6. Using the centering screws on the condenser hanger ③, center the iris diaphragm in the field ④. The screws may have integrated thumbscrews or require a hex wrench.
7. Open the field diaphragm most of the way (e.g. you can still see the leaves in the field of view), and check the center.
8. Using the condenser focus know, adjust the condenser height so that the leaves of the field iris diaphragm are sharp. There may be some slight blue or orange coloration on the edge of the leaves.
9. Open the field diaphragm all the way.
10. For best results, repeat for the objective you will be using for imaging and manual slide scanning.



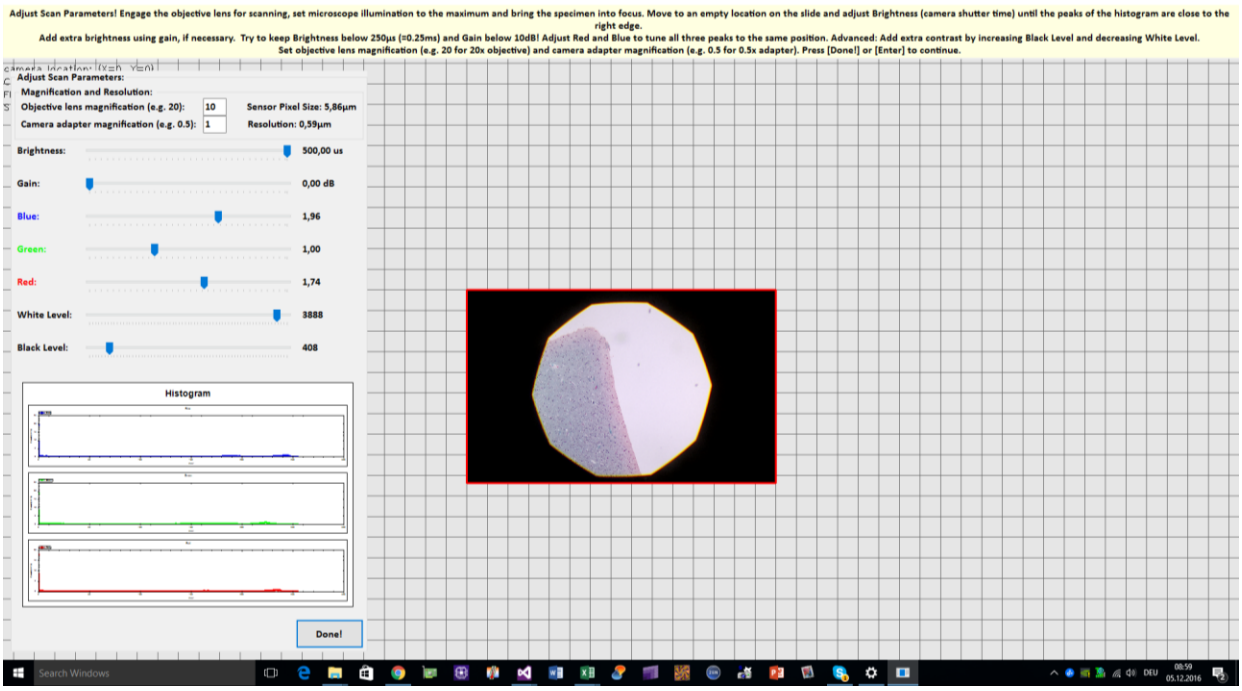




The alignment of the illumination can be checked easily after starting up the ACCU-*Slide*MS software.

After bringing the sample into focus, simply reduce the size of the field iris diaphragm until the image on the camera is reduced.

The screenshot below shows a well aligned illumination. The bright spot is in the center of the camera image and the edges of the field iris diaphragm are "sharp". If the image looks significantly different, please follow or repeat the procedure above to align Köhler illumination.




## Fine-Tuning the Camera Rotation

Manual slide scanning works best when the camera sensor is aligned to the X-Y motion directions of the stage.

When the camera is not well aligned well, the computer observes motion in both X and Y, even when moving the specimen in just one direction. Although the software can still successfully perform a scan, it is much more difficult for the operator to determine and follow the track of the scan. Camera/stage movement alignment can be easily seen in the software by observing the motion of the grid lines while moving the slide.

Fine-tuning the camera rotation can save scanning time, because it allows to reduce the amount of overlap between the stripes. In theory, an overlap greater than one grid tile between the current row/stripe and the last row/stripe should be enough when acquiring horizontal stripes. The vertical component of the motion results in reducing the overlap below one row of tiles. This results in stitching errors and bad image quality. Therefore, the overlap should be at least two or three rows of tiles to ensure proper stitching of the images.

Before rotating the camera, check that the stage is secured in position. Some stages have some “rotatability” via a locking screw in the front. Ensure that the stage is seated onto the stage carrier, and the locking screw is tightened.

Rotation of the camera can be tricky. Note that the camera should be mounted tightly on the camera adapter and cannot itself be rotated. Instead, rotate the camera by rotating the camera adapter in the camera port on the microscope.

The camera adapter usually is fixed using a locking screw. Adjust the rotation in very small steps, checking the movement against the grid pattern in the software. Be careful when tightening the screw so as not to alter the rotation.

As mentioned above, some microscopes have rotatable stages. On these microscopes you can adjust the rotation by rotating the stage too, however camera and stage alignment is generally performed more effectively by rotating the camera adapter.

## Preferred Alignment Method

**Adjust camera rotation by aligning the camera (or stage) to the ground edge of a slide.**

1. Choose a slide with excellent ground edges (no chipping). An empty slide can also be used for this procedure.
2. Clean the slide and slide holder to remove all dust.
3. Place the slide onto the stage, confirming that it is fully engaged in the slide holder.
4. Using a low magnification objective lens (4x or 10x magnification), move to the upper edge of the slide.
5. Focus on the edge of the glass slide. If using a specimen slide, do not focus on the edge of the coverglass.
6. To improve observation of angular error, position the edge of the slide towards the top of the field of view.
7. Align the camera to the edge of the slide by rotating the camera adapter or the stage.
8. Move the slide from left to right and observe the motion of the slide's edge as it transitions across the field of view.
9. If the slide's edge does not move up and down significantly, the camera should be sufficiently aligned.
10. If the slide's edge moves/varies up or down significantly, there may be one or more of the following causes.
    a. If the edge is moving up AND down when the slide is moved, the slide may not be suitable for calibration. To resolve this issue, use another slide.
    b. If the edge is moving up OR down while the slide is moved, the slide holder of the stage could be misaligned to the stage. This can easily happen if the slide holder is secured to the stage by two screws. To resolve the issue, unmount the slide holder, clean the slide holder and the stage from dust and remount the slide holder, ensuring it is fully seated against the mounting screws to align it properly.

Watch a video demonstration on camera alignment using the edge of a slide.

## Alternative Alignment Method

As an alternative (precise, but difficult) alignment method to the preferred method described above, you may use the image stitching process of the ACCU-*Slide*MS software to align the slide.

1. Select a specimen with very long horizontal features for long translation in the X direction.
2. Engage a high magnification objective (e.g. 40x)
3. Prepare as if beginning a slide scan. Choose the left edge of the sample as the starting point.
4. Start scanning and continue to move the image to the right for approximately 10 camera fields of view.
5. Observe the motion of the horizontal grid lines. Did the specimen move upwards or downwards?
    a. If the specimen moved less than the size of one tile upwards or downwards, the rotation is satisfactorily tuned/aligned.
    b. If the specimen moves upwards more than one tile, the camera needs to be rotated clockwise to reduce the drift.
    c. If the specimen moves downward more than one tile, the camera needs to be rotated counter-clockwise to reduce the drift.
6. Press F10 to reset the scan and repeat the procedure until condition 5.a. is satisfied.

# Scanning slides

## Preparation

**1. Quality of the specimen**

For manual whole slide imaging, it is important that the specimen is of good quality. Avoiding thickness variations of the specimen speeds up the scanning process by reducing the need to refocus while scanning.

**2. Structured scanning**

For optimal efficiency, plan the starting point and direction of the scan. Observe the sample at low magnification and memorize its contour. Don't lose orientation while scanning a specimen.

When scanning in horizontal stripes from top to bottom, locate the uppermost edge of the specimen and use it as starting point.

For difficult specimens with disjointed sample areas, simply take a picture of the whole slide with a smartphone first. This helps you to remember all areas that need to be scanned.

This virtual slide shows a difficult specimen. The disjointed areas were scanned in a counter-clockwise order. https://easyzoom.com/image/93238

**3. Warm up the halogen illumination**

Warming up the illumination is a crucial step for halogen light sources. Color and intensity of the lamp changes significantly in the first few minutes of operation before stabilizing.

If the brightness and color keep changing after a few minutes, this may be an indication to replace the bulb.

**4. Free up resources of your computer**

ACCU-*Slide*MS is hard work for your computer. To ensure that your computer has all its available resources to operate the scanning software, be sure to close all other applications before starting the ACCU-*Slide*MS application.

If using a portable (laptop) PC, attach the power supply before scanning.

Be patient while your computer completes the startup process. After booting your computer and logging into your Windows account, the computer requires at least one additional minute for the computers resources to be available. Running the ACCU-*Slide*MS software too early may cause unintended behavior.

## Basic Scanning

After setting up the scanning parameters, the next step is to prepare for scanning.

1. Press Done button or hit the Enter button to close the scanning parameters window.

## Shading Correction

1. To generate an even field of illumination (no shading), the software needs to "see" an empty field of the slide in order to estimate the background for shading correction. When starting your imaging within a tissue section, the specimen may appear very bright – this is normal as the software interprets the dark tissue/stain as shading and attempts to correct it. The software is designed to automatically update the shading correction.
2. To set the initial shading correction, move the sample out of the camera's field of view for a second, then move it back to a position on the edge of the specimen. The specimen should appear evenly illuminated.
3. Sometimes the shading-corrected image appears with errors, and these are generally caused by diffraction patterns within the sample. In these situations, simply move the sample out of the field of view and reset the shading correction by clicking the i-button ("i" as in illumination) to disable the illumination correction. Click the i-button again to re-enable shading correction. Return the sample to the field of view and begin scanning/observation again.

## Scanning

4. Begin a scanning project at the edge of the slide. At least 80% of the camera image should be covered by tissue.
5. Click enter on the keyboard to start scanning. The border around the live camera image turns green to indicate that the scanning is active and working.
6. Move the slide to acquire horizontal or vertical stripes. If your camera has a very wide aspect ratio (e.g. 16:10) it is recommended to scan vertical stripes (this speeds the acquisition). For cameras with standard aspect ratios (5:4 or 4:3), vertical and horizontal stripes are equally efficient.
7. Be sure to overlap adjacent stripes by AT LEAST to rows (or columns, tiles) of grids.
8. If the scanner loses track of its position (usually because of an absence of specimen material for stitching, or the scanning is too fast for the camera), the border of the live camera image turns red. Slowly return to the last known position and the software will "catch up".
9. Press F7 to save a scan to disk. or press F8 to save it to disk and upload it to a cloud service afterwards.

Watch this demonstration video of basic slide scanning.

## Freeze Feature

ACCU-*Slide*MS allows you to scan multiple areas of tissue on a single slide and combine them into one virtual slide.

Some slides contain multiple specimens that are not connected but should be scanned into one virtual slide. When there are enough particles in the empty parts of the slide, it is possible to move to the next piece of tissue.

For slides with areas devoid of tissue or particles, this is not possible because the scanning software loses its reference or position. In these situations, the scanning process can be “frozen”, the slide moved to a new location, and scanning resumed. Additionally, the corresponding starting position can be chosen in the virtual slide.

This feature allows arrangement the sections on the virtual slide differently than on the real slide. It is also possible to scan multiple real slides into one virtual slide.

After scanning the first tissue section, perform these steps to add another tissue section to the virtual slide:

1. Press "F" to enter Freeze-Mode. All image content that has already been scanned is frozen.
2. Move to the new starting location on the physical slide.
3. Using the mouse, drag the already-scanned image to another area of the virtual slide. To avoid overlapping the new scan with the already-scanned image, move the already-scanned image to a position on the virtual slide relative to its true position on the physical slide. Other locations may be freely chosen provided care is taken to avoid overlaps between the old and the new scan on the virtual slide.
4. Press Enter to start scanning or press Backspace to undo freezing.

Watch this demonstration video.