 nanocellect Biomedical, Inc.	WORK INSTRUCTION
Version 1.0	Title: Camera Tilt Assessment

1.0 Purpose: To provide step by step instructions on how to determine whether the camera is tilted.

2.0 Reference Material related to this work instruction:

- [Procedure WOLF-N1 01 Installation QC](#) or [Procedure WOLF-N1 02 Demo QC](#)
- [Procedure WOLF Troubleshooting Rainbow Bead Resolution Issues](#)
- [Procedure WOLF_07 Camera Adjustment](#)

3.0 Additional Tools Required:

- Camera Alignment Cartridge (PN#310126)
- ImageJ Software

4.0 Background Information:

Camera tilt can lead to a variety of downstream issues ranging from chip alignment failures to rainbow bead resolution issues. Camera tilt can be visualized by eye by assessing the live LED image of the chip center. However, if a cartridge appears to be tilted, do **NOT** assume that the camera is tilted. Due to cartridge manufacturing process, the microchip could be slightly tilted. To assess whether the camera is tilted, a specialized cartridge, the **camera alignment cartridge (PN#310126)**, must be used. This specialized cartridge has been validated at NanoCellec Headquarters to be perfectly straight. if the camera alignment cartridge appears to be tilted, then we can confidently presume that the camera is tilted.

This procedure can be performed at any point if there is doubt/concern about the camera alignment. When machines are brought up at NanoCellec Headquarters, camera tilt is assessed. Upon initial receipt and installation of a WOLF, the camera should be straight. A camera shift/tilt could occur if any of the four screws securing the camera in place loosen over time. However, the most common way to induce camera tilt is by performing a camera adjustment, according to "[Procedure WOLF_07 Camera Adjustment](#)". If the camera was adjusted, then this procedure **MUST** be completed to ensure that the camera was **NOT** accidentally tilted during the adjustment process.

5.0 Camera tilt assessment using the camera alignment cartridge:

5.1. Insert the **camera alignment cartridge** (PN#310126) into the WOLF.

Note: this work instruction MUST be performed with the camera alignment cartridge. It can NOT be performed with a sorting cartridge or the instrument specific reference cartridge.

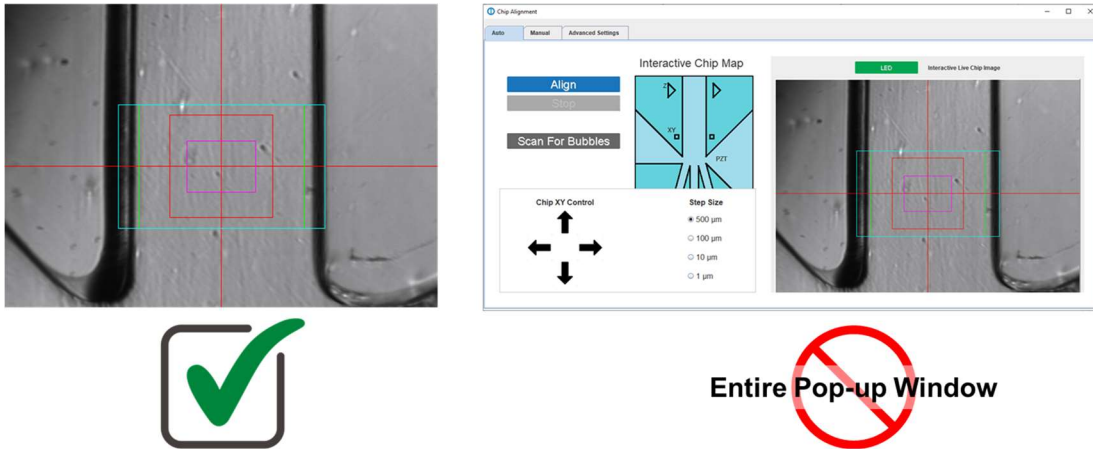
5.2. Align the cartridge by pressing the "Align" button within the "Auto" tab of the Chip Alignment pop-up window.

5.3. Once aligned, capture a screenshot of the live camera image only, NOT the entire Chip Alignment pop-up window (**Figure 1**).

Note: the screenshot should only encompass the chip center image, and NOT the chip map, control arrows etc.

Figure 1. Acquire screenshot of aligned camera alignment cartridge.

When acquiring a screenshot of the chip center of the camera alignment cartridge, make sure to only acquire the live camera image, not the entire Chip Alignment pop-up window.



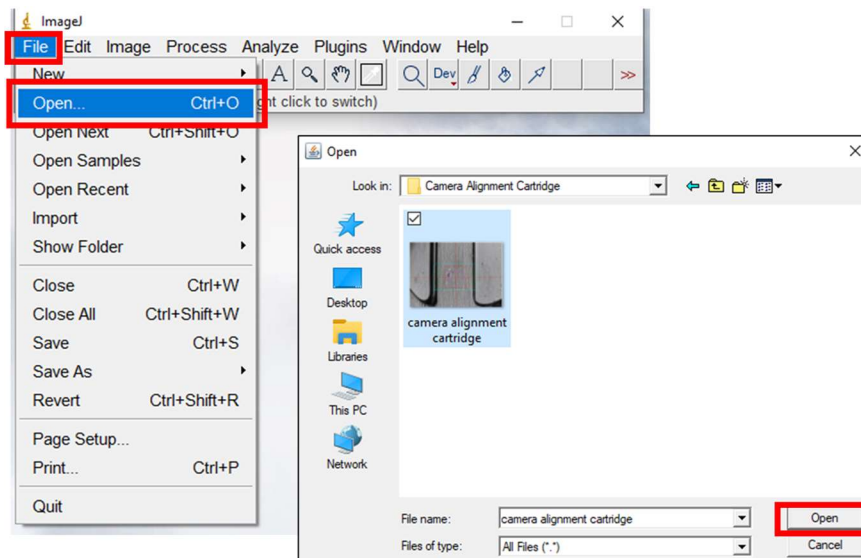
5.4. Save the image.

5.5. Open ImageJ software.

5.6. Within ImageJ, open the image file by selecting “Open” under the “File” tab on the menu bar. Within the resulting pop-up window, select the appropriate image file (**Figure 2**).

Figure 2. Opening image files on ImageJ

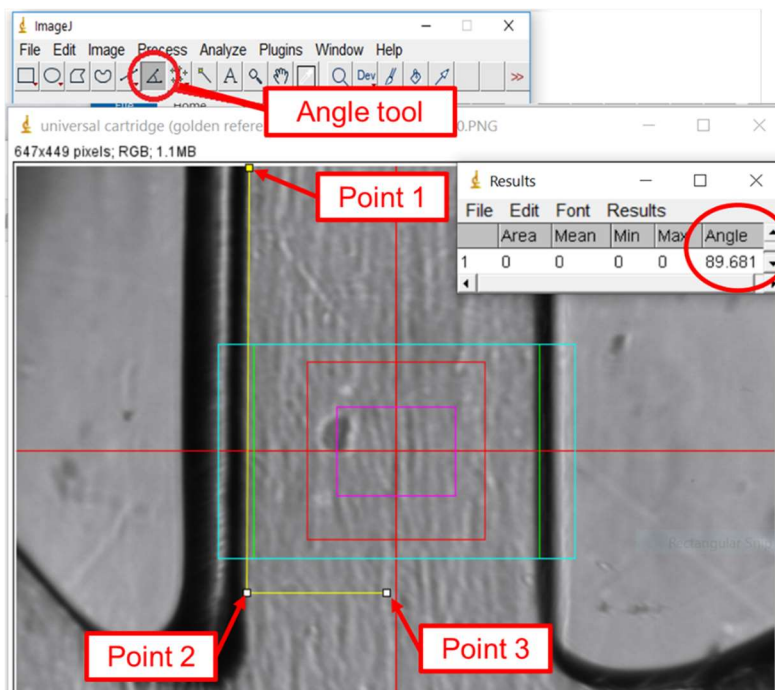
Select “Open” under the “File” tab on the menu bar to open the image of the chip center in ImageJ.



- 5.7. Select “Angle tool” and verify the tilt by measuring the following angle (**Figure 3**):
- 5.7.1. Move cursor to the top left side of the center channel, left click (**Figure 3- point 1**).
 - 5.7.2. Drag the yellow line vertically down the inside of the left center channel wall, parallel to the black edge, until reaching the bottom. Left click to set the reference point 2 (**Figure 3-point 2**).
 - 5.7.3. Carefully drag the yellow line to the right, parallel to the bottom edge of the image. Left-click to set the reference point 3 (**Figure 3-point 3**).
 - 5.7.4. Click “analyze” in the top main menu and select “measure”, or press “ctrl M”.
- 5.8. To calculate the tilt, subtract the measured angle value from 90.
- 5.9. Record tilt angle and compare with the Laser Alignment Report value. **Maximum acceptable tilt: +/- 1.3 degrees.**
- 5.10. If the tilt value meets acceptable criteria, then the camera is deemed straight.
- 5.11. If the tilt is greater than the acceptable criteria, then proceed to “**Procedure WOLF_07 Camera Adjustment**” to re-adjust the camera.

Figure 3. Utilize ImageJ to assess cartridge tilt of camera.

Import the image of the camera alignment cartridge into ImageJ to measure the degree of tilt of the camera, using the following three reference points.



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6.0 Revision History:

	ISSUED DATE	Description of Change	Author
A	12/09/2019	Document created	A.Bartakova
B	12/15/2019	New version – changed wording based on Beth Leary’s suggestions	A.Bartakova
C	04/24/2020	New version – added angle measurement workflow to avoid camera tilt. Added guidelines for movement in the Y axis. Reviewed by Manna Doud on 04/26/2020.	A. Bartakova
D	06/15/2020	New version edited for clarity. Figure representing a COC universal cartridge added.	A. Bartakova
E	09/28/2020	Reviewed; see comments/track changes	B. Desai
F	10/21/2020	New version edited for clarity. Figures added.	E. Rubio de la Torre
G	01/05/2021	V1.0 - Edited/Reduced/Created independent version separate from camera adjustment procedure. Goal was to increased compatibility with the Procedure for Troubleshooting Optical Misalignment	E. Leary