

ImageXpress Pico

Automated Cell Imaging System with CellReporterXpress Software

Calibration Kit Guide



ImageXpress Pico Automated Cell Imaging System Calibration Kit Guide

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Chapter 1: ImageXpress Pico Automated Cell Imaging System



The ImageXpress® Pico Automated Cell Imaging System is an all-in-one platform for automatically acquiring and analyzing images from fluorescently labeled biological samples in plates and slides. It enables you to increase the throughput of your image acquisition and analysis, allowing you to gain insights in minutes. With the addition of a modular option, the system provides environmental control for live cell imaging.

The core hardware component of the imaging system is a custom-designed, fully automated, epi-illumination fluorescence microscope. The rapid autofocus and precision sample movement features of the microscope allow large numbers of high-resolution images to be acquired in the shortest possible time. All key optical and mechanical elements are motorized, which allows complete control of the instrument configuration.

When used in combination with the CellReporterXpress® Image Acquisition and Analysis Software, which features powerful image analysis capabilities and more than 25 available predefined experimental protocols, the ImageXpress Pico system becomes an extremely flexible device, ideally suited for user-defined, automated assays.

Key components of the instrument include the following:

- Built-in, internal light source comprised of six high-powered LEDs enables very high sensitivity fluorescent imaging. Additional LED-based light sources allow transmitted light and overview imaging.
- High-sensitivity, 5-megapixel CMOS camera.
- Hardware-based autofocus system with precision motorized Z-stage focus.
- High-transmission fluorescence imaging optics with world-class chromatic aberration correction, resolution, and image flatness.
- Secondary camera provides overview imaging for slides.
- Precision motorized sample (X-Y) stage.
- High-quality Leica objectives in a six-position turret.
- Filter cubes in a six-position turret.
- Motorized selection of stage position, filter cubes, and objectives.
- Temperature control up to 40°C (104°F) for live cell imaging.
- Optional environmental control system, which enables you to regulate humidity, CO₂, and
 O₂ inside the environmental control cassette for multi-day, live-cell, time-lapse imaging.
- Operation and configuration control by the integrated CellReporterXpress software.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website—www.moleculardevices.com/service-support—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Technical Support

To contact Molecular Devices Technical Support, submit a support request through the Molecular Devices Knowledge Base at support.moleculardevices.com.

You can also submit a support request by phone. For regional support contact information, go to www.moleculardevices.com/contact.

To expedite support, please be prepared to provide the instrument serial number. The serial number is located on the back panel of the instrument.



Documentation

Review the product documentation on the Molecular Devices Knowledge Base at support.moleculardevices.com, including installation guides and user guides. In addition, online Help is available within the CellReporterXpress software. Press **F1** to access Help for the current page.

Additional Resources

Web-based microscopy courses:

- www.leica-microsystems.com/science-lab
- www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-short-course.html

The Molecular Probes Handbook offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis:

www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook.html

Product Documentation

The following guides are available on the Molecular Devices Knowledge Base at support.moleculardevices.com:

- CellReporterXpress Installation & IT Guide
- CellReporterXpress User Guide
- CellReporterXpress Release Notes
- ImageXpress Pico Pre-Installation Guide
- ImageXpress Pico EC Gas Requirements Pre-Installation Guide
- ImageXpress Pico Product Safety Sheet
- ImageXpress Pico Installation Guide
- ImageXpress Pico User Guide
- ImageXpress Pico Calibration Kit Guide

In addition, the CellReporterXpress software includes context-sensitive Help that you can access from within the software. Just press the **F1** key from within the software to view Help for the current page.



Tip: We recommend that you review the documentation before installing or using the ImageXpress Pico system or the CellReporterXpress software.

About This Guide

This guide is intended for the scientist who receives a calibration kit along with the aftermarket purchase of an objective or filter cube for the ImageXpress Pico system. It describes how to install and calibrate the objective or filter cube.

The information in this guide is subject to change without notice. We recommend that you review the guide on the Molecular Devices Knowledge Base at support.moleculardevices.com for the most up-to-date information.

Chapter 2: Package Contents



The Calibration Kit includes the following:

| ltem | Part number | Description |
|---|----------------|------------------------|
| | 5063669 | Calibration Kit Guide |
| WRECOUR ERVERS | 9100-0099 | Fluorescent Bead Slide |
| THE FACE COMME | 1-GP-7 | Red Plastic Slide |
| THE PACE DOWN GP — 8 White of it beauty | 1-GP-8 | Green Plastic Slide |
| one dest down | 1-GP-11 | Yellow Plastic Slide |

For a complete list of the contents of the package, see the enclosed packing list.

Chapter 3: Filter Cube Maintenance



Filter cube maintenance procedures include the following:

- Installing a Filter Cube, see below
- Calibrating a Filter Cube, see page 15

Installing a Filter Cube

Before installing a filter cube, review the following:

- Access only the user-serviceable components inside the instrument as described in this guide. Avoid contact with other components as they can be damaged or knocked out of alignment.
- To prevent dust from collecting inside the instrument, keep the top door and the maintenance door closed (unless you are inserting labware or performing maintenance tasks).
- Ensure that the top door and the maintenance door are closed before starting the instrument.



CAUTION! To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.

The following filter cubes are available:

- DAPI
- CFP
- FITC
- TRITC
- Texas Red
- Cy5



Note:

- Depending on how you have configured your ImageXpress Pico system, you may not have received all the filter cubes.
- Do not install a filter cube in slot 6 of the turret. Slot 6 must be empty (in both the instrument and the software).

Installing a Filter Cube in an Empty Slot

To install a filter cube in an empty slot:



- 1. In the CellReporterXpress software, on the Home page, click
- 2. Click Show Device Options to expand the details for the device where you want to install a filter cube.
- 3. Click the Filters tab.
- 4. In the tile for the filter cube slot where you want to install, click Component Exchange.



Note: Do not install a filter cube in slot 6 of the turret. Slot 6 must be empty (in both the instrument and the software).

- 5. Click the Choose Filter drop-down list box, and select the filter cube you want to install.
- 6. Click Open Maintenance Door.
- 7. If needed, slightly rotate the filter cube turret by hand to get direct access to the filter cube slot.
- 8. Install the filter cube in the slot by gently pushing it into the slot until it "snaps" into place.



CAUTION! Retain the filter cube packaging. When not installed in the instrument, a filter cube should always be stored in its original packaging.

- 9. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click Close Maintenance Door.
- 10. Click Apply.

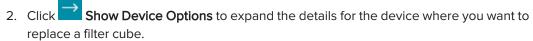
After you install a new filter cube from an after-sales purchase, you must calibrate it. See Calibrating a Filter Cube on page 15 for details.

Replacing a Filter Cube (Different Wavelength)

To replace a filter cube with a filter cube of a different wavelength:



1. In the CellReporterXpress software, on the Home page, click



- 3. Click the Filters tab.
- 4. In the tile for the filter cube slot where you want to install, click **Component Exchange**.
- 5. Click the **Choose Filter** drop-down list box, and select the filter cube you want to install.
- 6. Click Open Maintenance Door.
- 7. If needed, slightly rotate the filter cube turret by hand to get direct access to the filter cube slot.
- 8. Remove the filter cube to be replaced from the instrument by gently pulling it toward you.



CAUTION! When not installed in the instrument, a filter cube should always be stored in its original packaging.

9. Install the filter cube in the slot by gently pushing it into the slot until it "snaps" into place.



CAUTION! Retain the filter cube packaging for future storage needs. When not installed in the instrument, a filter cube should always be stored in its original packaging.

- 10. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
- 11. Click Apply.

After you replace a filter cube, you may need to calibrate it. See Calibrating a Filter Cube on page 15 for details.



Note: If you re-install the same filter cube (after cleaning it, for example), you do not need to calibrate it.

Replacing a Filter Cube (Same Wavelength)

The typical reason to replace a filter cube with another filter cube of the same wavelength is when you are replacing a damaged filter cube.

To replace a filter cube with a filter cube of the same wavelength:



- 1. In the CellReporterXpress software, on the **Home** page, click
- 2. Click Show Device Options to expand the details for the device where you want to replace a filter cube.
- 3. Click the Filters tab.
- 4. In the tile for the filter cube slot where you want to replace, click **Component Exchange**.
- 5. Click the **Choose Filter** drop-down list box, and select **None**.
- 6. Click Open Maintenance Door.
- 7. If needed, slightly rotate the filter cube turret by hand to get direct access to the filter cube slot.
- 8. Remove the filter cube to be replaced from the instrument by gently pulling it toward you.



CAUTION! When not installed in the instrument, a filter cube should always be stored in its original packaging.

9. Install the filter cube in the slot by gently pushing it into the slot until it "snaps" into place.



CAUTION! Retain the filter cube packaging for future storage needs. When not installed in the instrument, a filter cube should always be stored in its original packaging.

- 10. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
- 11. Click Close.

After you replace a filter cube, you may need to calibrate it. See Calibrating a Filter Cube on page 15 for details.



Note: If you re-install the same filter cube (after cleaning it, for example), you do not need to calibrate it.

Calibrating a Filter Cube

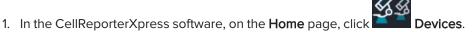
After you install a new filter cube from an after-sales purchase, you must calibrate it. A calibration kit, which is included with any after-sales filter cube purchase, includes the following:

- Fluorescent Bead Slide (9100-0099)
- Red Plastic Slide (1-GP-7)
- Green Plastic Slide (1-GP-8)
- Orange Plastic Slide (1-GP-11)

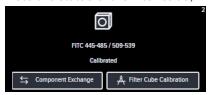
You will need to provide the following item for the calibration process:

• 4-Slide Holder (5068503)

To calibrate a filter cube:



- 2. Click Show Device Options to expand the details for the device where you want to calibrate a filter cube.
- 3. Click the Filters tab.
- 4. Note the status of the filter cube, which is indicated in the tile.



- If the tile indicates **Not Calibrated**, continue to the next step to perform the calibration procedure.
- If the tile indicates **Calibrated**, you typically do not need to perform the calibration procedure and you can skip this procedure.



Note: An exception to this is when you replace a filter cube with the same type of filter cube. In this case, you should continue to the next step and perform the calibration procedure regardless of the label in the tile.

5. In the tile for the filter cube you want to calibrate, click Filter Cube Calibration.

6. Follow the on-screen instructions to complete the calibration.



Tip:

- In the Choose Slide Holder drop-down list box, select 4 Slide Holder.
- Insert the slides face down in the slide holder (that is, printed side down).
- When each step completes, click **Next** to continue to the next step.

Chapter 4: Objective Maintenance



Objective maintenance procedures include the following:

- Installing an Objective, see page 18
- Calibrating an Objective, see page 22
- Adjusting an Objective Correction Collar, see page 23
- Cleaning an Objective, see page 27

You can identify the magnification of an objective by the color band:

| Objective Magnification | Color Band |
|-------------------------|------------|
| 4x | Red |
| 10x | Yellow |
| 20x | Green |
| 40x | Light Blue |
| 63x | Dark Blue |



The standard objectives in the ImageXpress Pico system are configured and calibrated by Molecular Devices when the instrument is delivered.



CAUTION! Replace objectives in their original positions.



Tip: We recommend removing and maintaining only one objective at a time.

Installing an Objective

Before installing an objective, review the following:

- Access only the user-serviceable components inside the instrument as described in this guide. Avoid contact with other components as they can be damaged or knocked out of alignment.
- To prevent dust from collecting inside the instrument, keep the top door and the maintenance door closed (unless you are inserting labware or performing maintenance tasks).
- Ensure that the top door and the maintenance door are closed before starting the instrument.



CAUTION!

- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.

Molecular Devices precalibrates the objectives to specific slots in the turret. You must install the objectives as follows:

| Slot | Objective Magnification |
|------|-------------------------|
| 1 | 4x |
| 2 | 10x |
| 3 | 20x |
| 4 | empty |
| 5 | 40x or 63x |
| 6 | empty |



Note:

- Depending on how your ImageXpress Pico system is configured, you may not have all the objectives.
- Do not install the 40x objective and the 63x objective in the instrument simultaneously.
- The 63x objective is not compatible with the environmental control cassette. Do not install the 63x objective when the environmental control cassette is loaded.
- If no filter cubes are installed, an installed objective will appear to be not installed.
 We recommend that at least one filter cube be installed before installing an objective. See Installing a Filter Cube on page 11 for details.

Installing an Objective in an Empty Slot

You must install objectives in specific slots in the turret. See Installing an Objective on page 18 for details.

To install an objective in an empty slot:



- 1. In the CellReporterXpress software, on the **Home** page, click
- 2. Click Show Device Options to expand the details for the instrument where you want to install an objective.
- 3. Click the **Objectives** tab.
- 4. In the tile for the objective slot where you want to install, click Component Exchange.
- 5. Click the **Choose Objective** drop-down list box, and select the objective you want to install.
- 6. Click Open Maintenance Door.
- 7. Install the objective in the slot by gently turning it clockwise.





CAUTION! Retain the objective case. When not installed in the instrument, an objective should always be stored in its case.

- 8. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
- 9. Click Apply.

After you install a new objective from an after-sales purchase, you must calibrate it. See Calibrating an Objective on page 22 for details.

Replacing an Objective (Different Magnification)

You must install objectives in specific slots in the turret. See Installing an Objective on page 18 for details. Because of this, the only reason to replace an objective with another objective of a different magnification is when you are a swapping the 40x objective and the 63x objective in slot 5.

To replace an objective with an objective of a different magnification:



- 1. In the CellReporterXpress software, on the **Home** page, click
- 2. Click Show Device Options to expand the details for the instrument where you want to replace an objective.
- 3. Click the **Objectives** tab.
- 4. In the tile for objective slot **5**, click **Component Exchange**.
- 5. Click the **Choose Objective** drop-down list box, and select the objective you want to install.
- 6. Click Open Maintenance Door.
- 7. Remove the objective to be replaced from the instrument by gently turning it counterclockwise.



CAUTION! When not installed in the instrument, an objective should always be stored in its case.

8. Install the replacement objective in the slot by gently turning it clockwise.



CAUTION! Retain the objective case for future storage needs. When not installed in the instrument, an objective should always be stored in its case.

- 9. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click Close Maintenance Door.
- 10. Click Apply.

After you replace an objective, you may need to calibrate it. See Calibrating an Objective on page 22 for details.

Replacing an Objective (Same Magnification)

You must install objectives in specific slots in the turret. See Installing an Objective on page 18 for details. The typical reason to replace an objective with another objective of the same magnification is when you are replacing a damaged objective.

To replace an objective with an objective of the same magnification:



- 2. Click Show Device Options to expand the details for the instrument where you want to replace an objective.
- 3. Click the **Objectives** tab.
- 4. In the tile for the objective slot where you want to install, click Component Exchange.
- 5. Click the **Choose Objective** drop-down list box, and select **None**.
- 6. Click Open Maintenance Door.
- 7. Remove the objective to be replaced from the instrument by gently turning it counterclockwise.



CAUTION! When not installed in the instrument, an objective should always be stored in its case.

8. Install the replacement objective in the slot by gently turning it clockwise.



CAUTION! Retain the objective case for future storage needs. When not installed in the instrument, an objective should always be stored in its case.

- 9. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click Close Maintenance Door.
- 10. Click Close.

After you replace an objective with another objective, you may need to calibrate it. See Calibrating an Objective on page 22 for details.



Note: If you re-install the same objective (after cleaning it, for example), you typically do not need to calibrate it.

Calibrating an Objective

After you install a new objective from an after-sales purchase, you must calibrate it. A calibration kit, which is included with any after-sales objective purchase, includes the following:

- Fluorescent Bead Slide (9100-0099)
- Red Plastic Slide (1-GP-7)
- Green Plastic Slide (1-GP-8)
- Orange Plastic Slide (1-GP-11)

You will need to provide the following item for the calibration process:

• 4-Slide Holder (5068503)

To calibrate an objective:



- 2. Click Show Device Options to expand the details for the device where you want to calibrate an objective.
- 3. Click the **Objectives** tab.
- 4. Note the status of the objective, which is indicated in the tile.



- If the tile indicates **Not Calibrated**, continue to the next step to perform the calibration procedure.
- If the tile indicates **Calibrated**, you typically do not need to perform the calibration procedure and you can skip this procedure.



Note: An exception to this is when you replace an objective with another objective of the same magnification. In this case, you should continue to the next step and perform the calibration procedure regardless of the label in the tile.

- 5. In the tile for the objective you want to calibrate, click **Objective Calibration**.
- 6. Follow the on-screen instructions to complete the calibration.



Tip:

- In the Choose Slide Holder drop-down list box, select 4 Slide Holder.
- Insert the slides face down in the slide holder (that is, printed side down).
- When each step completes, click **Next** to continue to the next step.

Adjusting an Objective Correction Collar

The 40x objective and 63x objective have application-optimized correction collars to compensate for well bottom thickness or coverslip thickness. The collars have a range of 0 mm to 2 mm correction. Changing this setting adjusts the distances between components inside the objective barrel. Image quality and resolution are very dependent on properly setting these collars.



The settings to be used depend on the well bottom thickness of the plate or the coverslip thickness on the slide on which the specimen is mounted. In general, set the correction collar for the physical thickness of the plate or slide that you are imaging. The physical thickness can be determined by the plate specifications from the plate manufacturer.



Note: Do not use a plate, slide, or coverslip with a thickness that is out of the range of the correction collar for the selected objective.

Before adjusting an objective correction collar, review the following:

- Access only the user-serviceable components inside the instrument as described in this guide. Avoid contact with other components as they can be damaged or knocked out of alignment.
- To prevent dust from collecting inside the instrument, keep the top door and the maintenance door closed (unless you are inserting labware or performing maintenance tasks).
- Ensure that the top door and the maintenance door are closed before starting the instrument.



CAUTION!

- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.

You would typically adjust a correction collar as part of setting up an acquisition.

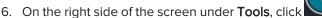
Adjusting an Objective Correction Collar for a Plate

To adjust an objective correction collar for a plate:



- 1. In the CellReporterXpress software, on the Home page, click
- 2. Click Add Protocol.
- 3. Click New Plate Acquisition.
- 4. In the Acquisition Devices list, select the instrument.
- 5. On the left side of the screen under **Steps**, click







7. In the **Plate Format** list, select the plate format.



- 8. On the right side of the screen under **Tools**, click **Obj**
- In the Objectives list, select the objective.
 If a correction collar adjustment is required, the software displays the recommended setting.

You may need to perform correction of objective collar for the current objective. Use value: 0.19



Note: When using the environmental control cassette with a 40x objective, you must adjust the objective correction collar an additional 0.7 mm above the recommended setting to account for the thickness of the cassette glass bottom. If the bottom thickness of the plate plus 0.7 mm is greater than the range of the 40x objective correction collar, then this plate is not compatible with this configuration. Try a different plate type, use a lower magnification objective, or image without the environmental control cassette.



10. On the left side of the screen under **Steps**, click

Acquisition Device.





Set Up for Adjustment of Objective Collar.

- 12. Click **OK**.
 - The maintenance door opens.
- 13. If needed, loosen the objective from the instrument by gently turning it counterclockwise.
- 14. Rotate the correction collar to its new setting.



Tip: You might need a flashlight to see the markings for the graduated scale on the barrel and its current setting.

15. If you loosened the objective, tighten it by gently turning it clockwise.



Note: When tightening the objective, take care to avoid changing the correction collar setting.

16. Close the maintenance door.



17. On the right side of the screen under **Tools**, click **Finish Adjustment of Objective Collar** to exit maintenance mode.

18. Click **OK**.

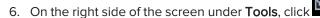
Adjusting an Objective Correction Collar for a Slide

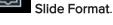
To adjust an objective correction collar for a slide:



- 1. In the CellReporterXpress software, on the Home page, click
- 2. Click Add Protocol.
- 3. Click New Slide Acquisition.
- 4. In the Acquisition Devices list, select the instrument.
- 5. On the left side of the screen under Steps, click







7. In the **Slide Format** list, select the slide format.



8. On the right side of the screen under **Tools**, click

9. In the **Objectives** list, select the objective. If a correction collar adjustment is required, the software displays the recommended setting.

You may need to perform correction of objective collar for the current objective. Use value: 0.19

10. On the left side of the screen under Steps, click



11. On the right side of the screen, click



- Set Up for Adjustment of Objective Collar.
- 12. Click **OK**.

The maintenance door opens.

- 13. If needed, loosen the objective from the instrument by gently turning it counterclockwise.
- 14. Rotate the correction collar to its new setting.



Tip: You might need a flashlight to see the markings for the graduated scale on the barrel and its current setting.

15. If you loosened the objective, tighten it by gently turning it clockwise.



Note: When tightening the objective, take care to avoid changing the correction collar setting.

16. Close the maintenance door.



Finish Adjustment of Objective 17. On the right side of the screen under Tools, click Collar to exit maintenance mode.

18. Click **OK**.

Cleaning an Objective

If debris and contaminants collect on an objective lens, you can clean it. Before cleaning an objective, review the following:

- Access only the user-serviceable components inside the instrument as described in this guide. Avoid contact with other components as they can be damaged or knocked out of alignment.
- To prevent dust from collecting inside the instrument, keep the top door and the maintenance door closed (unless you are inserting labware or performing maintenance tasks).
- Ensure that the top door and the maintenance door are closed before starting the instrument.



CAUTION!

- To prevent skin oils from damaging the optical coatings, we recommend that you wear powder-free disposable gloves when handling objectives and filter cubes.
- With the instrument power on, do not manually rotate the objective turret. Manually rotating the objective turret can damage the instrument.
- Do not use a product that disperses aerosol propellants or fluid onto the lens surface, such as canned compressed air.
- Do not use a wipe that can leave lint, such as Kimwipes.

To clean an objective:



- 1. In the CellReporterXpress software, on the **Home** page, click
- 2. In the **Available Acquisition Devices** list, select the instrument.
- 3. Click the **Objectives** tab.
- 4. In the tile for the objective you want to clean, click Component Exchange.
- 5. Click the Choose Objectives drop-down list box, and select None.
- 6. Click Open Maintenance Door.
- 7. Remove the objective from the instrument by gently turning it counterclockwise.
- 8. Place the objective on a secure surface away from the instrument
- 9. Use a bulb duster to carefully blow dust contaminants off the objective.
- 10. Use lens paper to gently wipe the objective free of contaminants.



Tip: If needed, you can use a 100% methanol lens cleaner. Refer to Leica for details on preferred cleansing solvent and procedure.

- 11. If needed, wait a few minutes for the methanol to evaporate.
- 12. Install the objective back its original slot in the turret by gently turning it clockwise.



Tip: If the objective has a correction collar, make sure that the collar is at the correct setting when reinstalling it.

- 13. Do both of the following:
 - a. Manually close the maintenance door.
 - b. In the CellReporterXpress software, click **Close Maintenance Door**.
- 14. Click Close.

After cleaning an objective, you may want to acquire a sample image. If image quality is degraded, repeat this procedure.

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