WESTERN DETECTION

# Stunningly easy western blot and gel imaging

# Colony counting with iBright Imaging Systems

## Introduction

To expand the application breadth of the Invitrogen™ iBright™ Imaging Systems platform, capabilities to image multiple cell culture plates (Figures 1–4) have been added to iBright Imaging Systems running firmware version 1.8* or higher. One can image blue, white, chemiluminescent, fluorescent, and crystal violet–stained colonies on single- or multi-well cell culture plates. A custom colony dye can also be created to support other colony imaging needs.

For convenience, in addition to colony imaging, there is an on-instrument analysis workflow for colony counting. Wells and colonies will automatically be detected using a proprietary detection algorithm, which streamlines the otherwise manual counting process. If desired, the default analysis output can be fine-tuned by adjusting the gating parameters to filter by colony size, intensity, and circularity to eliminate nonspecific colonies. To simplify documentation of the colony counting process, a report can be generated directly from the instrument and exported to an external drive or printed on a network printer. Image files can also be exported to an external drive or the cloud (Thermo Fisher™ Connect™ Platform) for storage. Files in G2i format can be imported into Invitrogen™ iBright™ Analysis Software for further analysis.

## How to capture a colony plate image

1. Place the plate on the turntable of an iBright Imaging System with the lid on and plate facing upwards.
2. Close the drawer and select **Universal Mode**, followed by the channel with the desired imaging conditions.
3. Select multiple channels if screening for total colonies, e.g., **Visible Blue Colonies** and **Visible White Colonies**.
4. **Capture** an image using the **Smart Exposure**™ automatic exposure feature for an optimized exposure time.
5. If analysis is desired, proceed to the analysis workflow on the instrument or export the image from the instrument, and then import the image into iBright Analysis Software for further analysis and annotation.

* iBright Imaging Systems firmware 1.8 was released on November 15, 2022.

**Figure 1. Imaging of competent cells transformed with GFP.** Cells were transformed using 10 ng of a plasmid expressing GFP. After the plasmid uptake step, 50 µL of cells were spread on 1.5% agar cell culture plates containing kanamycin. Plates were incubated overnight at 37°C to allow for proper growth. The image was captured in **Universal Mode** selecting the **Trans GFP** channel, using **Trans M2** filter sources on the Invitrogen™ iBright™ FL1500 system. If imaging of GFP colonies through epi-illumination is desired, select the **Epi GFP** channel.

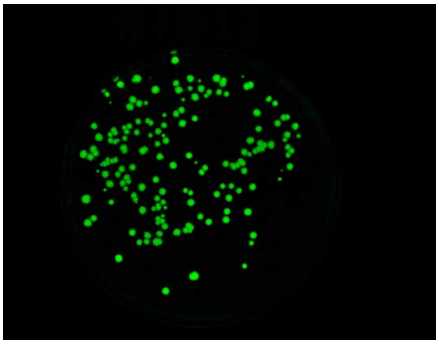

**Figure 2. False-color images for alternative visualization.** A green false color was applied to the image in Figure 1. A total of 14 false colors can be applied to colony count applications during on-instrument analysis or when using iBright Analysis Software after an image has been exported.

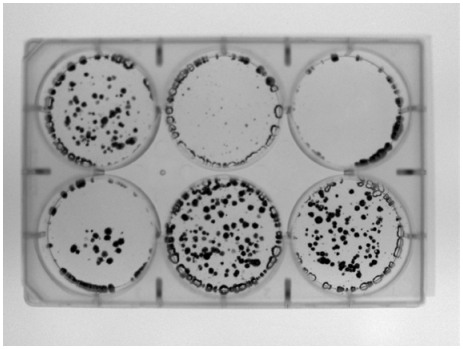

**Figure 3. Imaging of mammalian cell colonies.** A mammalian cell line was treated with a cell proliferation inhibitor at various concentrations. The inhibitor's efficacy was investigated through crystal violet staining of the colonies. The image was captured in **Universal Mode** selecting the **Visible Crystal Violet** channel, using **Trans M2** filter sources on the iBright FL1500 system. When imaging visible crystal violet colonies, a white screen or contrast tray is required for optimal imaging. If imaging crystal violet colonies through epi-illumination is desired, select the **Fluorescent Crystal Violet** channel and image without a tray.

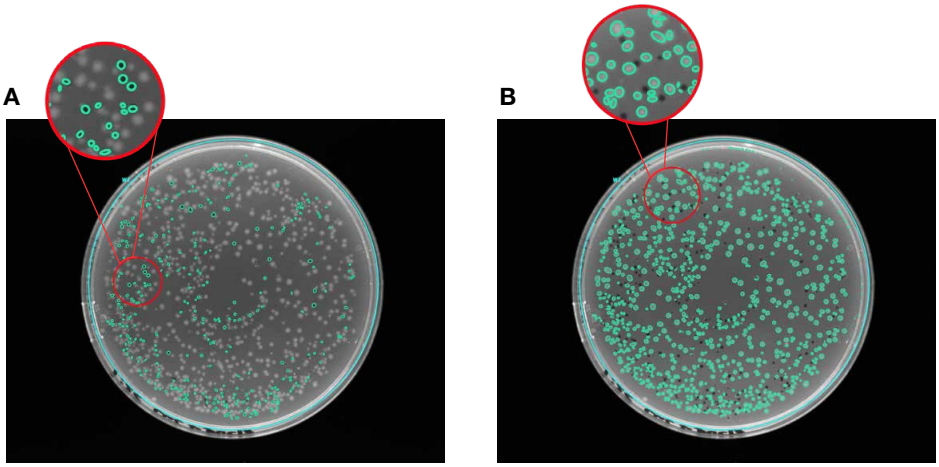


**Figure 4. Imaging of colonies transformed with vectors with and without the β-galactosidase gene.** The cells were transformed with a vector carrying a functional β-galactosidase gene and another vector lacking the β-galactosidase gene, to screen for blue and white colonies, respectively. The detected colonies are shown by green boundaries in these images. After the vector uptake step, 50 µL of cells were spread on 1.5% agar cell culture plates containing ampicillin, IPTG, and X-Gal. Plates were incubated overnight at 37°C to allow for proper growth. Images were captured in **Universal Mode** selecting the **Visible Blue Colonies** channel and the **Visible White Colonies** channel, using **Trans M2** filter sources on the iBright FL1500 system. Blue colonies **(A)** indicate expression of β-galactosidase activity, and white colonies **(B)** indicate absence of β-galactosidase activity.

**Table 1. iBright Imaging System filter recommendations for plate-based specialty imaging applications.**

| Recommended preset filter settings per sample type | | |
|---|---|---|
| **Sample type** | **Excitation channel** | **Emission channel** |
| **GFP*** | Trans (490–520 nm) | M2 (568–617 nm) |
| | Epi-X1 (455–485 nm) | M1 (508–557 nm) |
| **Crystal violet**** | Trans† (490–520 nm) | M2 (568–617 nm) |
| | Epi-X3 (608–632 nm) | M3 (675–720 nm) |
| **Visible blue colonies** | Trans (490–520 nm) | M2 (568–617 nm) |
| | Note: Black signal on white background setting selected | |
| **Visible white colonies** | Trans (490–520 nm) | M2 (568–617 nm) |
| | Note: White signal on black background setting selected | |

* Recommended filter selections are model dependent. Use excitation channel Trans (490–520 nm) and emission channel M2 (568–617 nm) for the Invitrogen™ iBright™ CL750 and CL1500 models, and excitation channel Epi-X1 (455–485 nm) and emission channel M1 (508–557nm) for the Invitrogen™ iBright™ FL1500 model.

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† A white screen or contrast tray is required when imaging crystal violet–stained colonies with transillumination.

## Tips to optimize colony counting applications

1. Avoid writing on the lid and the bottom of the plate, as this will interfere with detection of colonies. If needed, writing on the side of the plate is recommended (Figure 5).
2. Do not use epi-illumination when imaging with a white screen or contrast tray on the iBright Imaging System, as this will cause reflections visible in the image (Figure 6).
3. If the parallax effect is observed when imaging, use 1x zoom (i.e., no zoom), or turn the plate upside down if it does not contain liquid, to reduce the effect (Figure 7).
4. Culture plates that contain agar with varying thicknesses may require focus adjustments. If the image is out of focus, select **Auto focus** in the **More options** menu in live view, or manually adjust the focus (not shown).

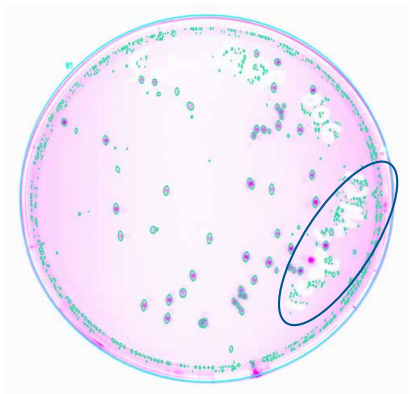

**Figure 5. False signal in colony counting.** Ink marker writing on the bottom of the plate caused observable false signals that interfered with accurate colony counting.

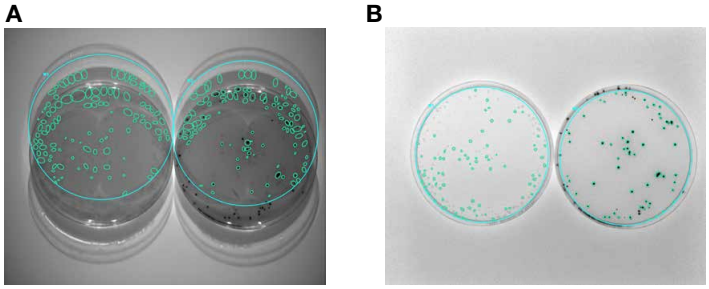


**Figure 6. Imaging with epi-illumination and transillumination.** **(A)** Undesirable reflections are observed when using epi-illumination in combination with a white screen. **(B)** Reflections are minimized when using transillumination.

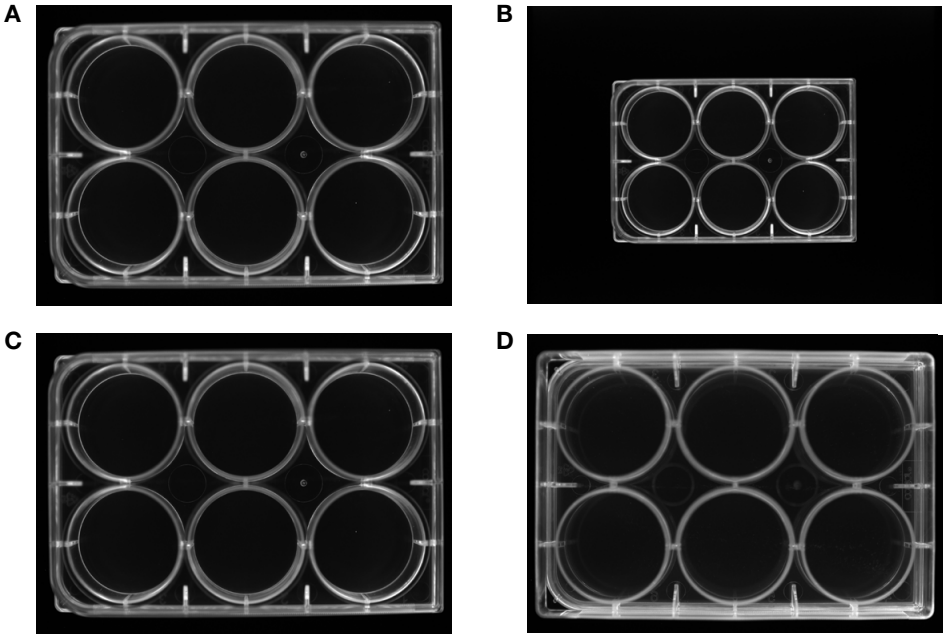


**Figure 7. Overcoming the parallax effect.** The parallax effect was reduced in **B** compared to **A** by reducing the zoom level from 1.6x zoom to 1x zoom (i.e., no zoom). The effect was reduced in **D** compared to **C** by turning the plate upside down.

## How to analyze colonies on iBright Imaging Systems

In the **Image Analysis** workflow, you can adjust gating, edit analysis wells, change channels, designate a background, or move to more options (Figure 8). Gating is well-specific and can be applied to three parameters (colony size, average pixel intensity, and circularity). For the **Colony Size** parameter, move the top slider down to remove large colony areas or the bottom slider up to remove small colony areas. For the **Average Pixel Intensity** parameter, move the top slider down to remove high-intensity pixels or the bottom slider up to remove low-intensity pixels. For the **Circularity** parameter, move the top slider down to remove more circular colonies or the bottom slider up to remove less circular colonies.

To adjust the analysis well, select one of the four handles of the well to be resized on the viewport and adjust using the (+) and (–) symbols or by touching the slider bars and dragging them with your finger. A well can be added by selecting **Add new well** or removed by selecting **Remove well**. If the correct detection of colonies is not being displayed, you can change the background designation to aid in proper colony detection (Figure 9).

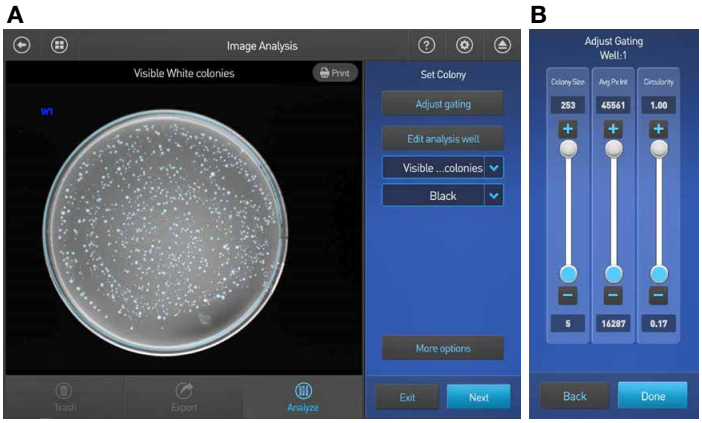


**Figure 8. An example of visible blue colony analysis with gate adjustment (A) using slider bars (B) to fine-tune the gating parameters.**

The bright edges of a cell culture plate can be falsely interpreted as nonspecific colonies. These can be removed by resizing the well to fit around the innermost edge of the culture plate, or by adjusting the gating for colony size and average pixel intensity to remove the falsely identified nonspecific colonies (Figure 10).

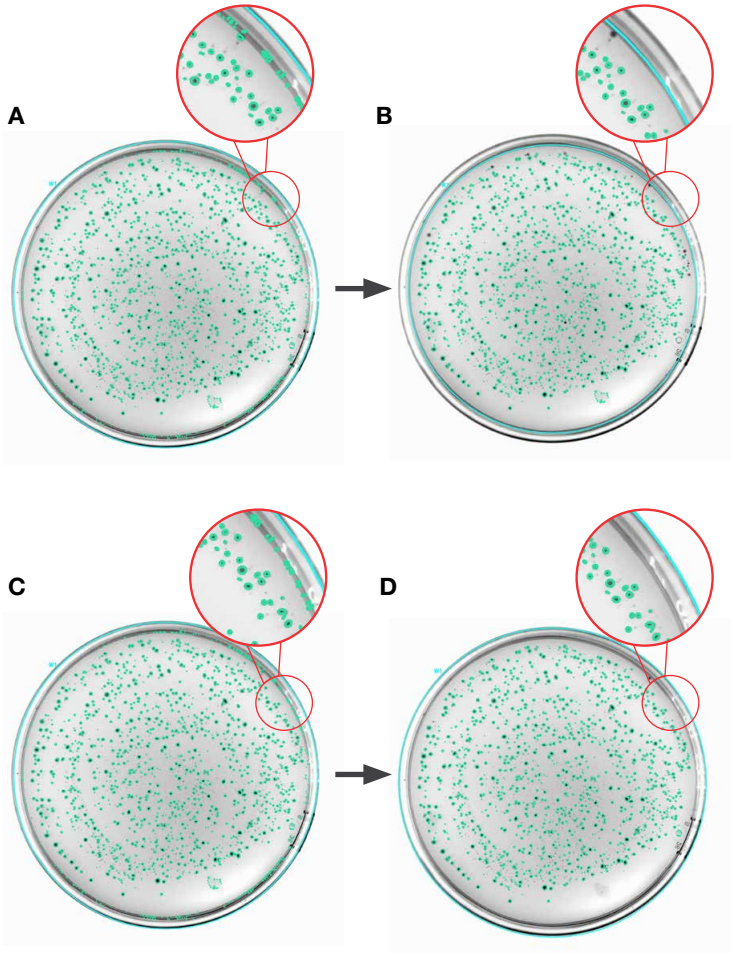


**Figure 10. Removal of nonspecific colonies.** Bright edges of a cell culture well or plate can be falsely interpreted as nonspecific colonies **(A, C).** Removal of falsely identified nonspecific colonies can be achieved by resizing the well as shown in **B** and by adjusting the gating as shown in **D**.

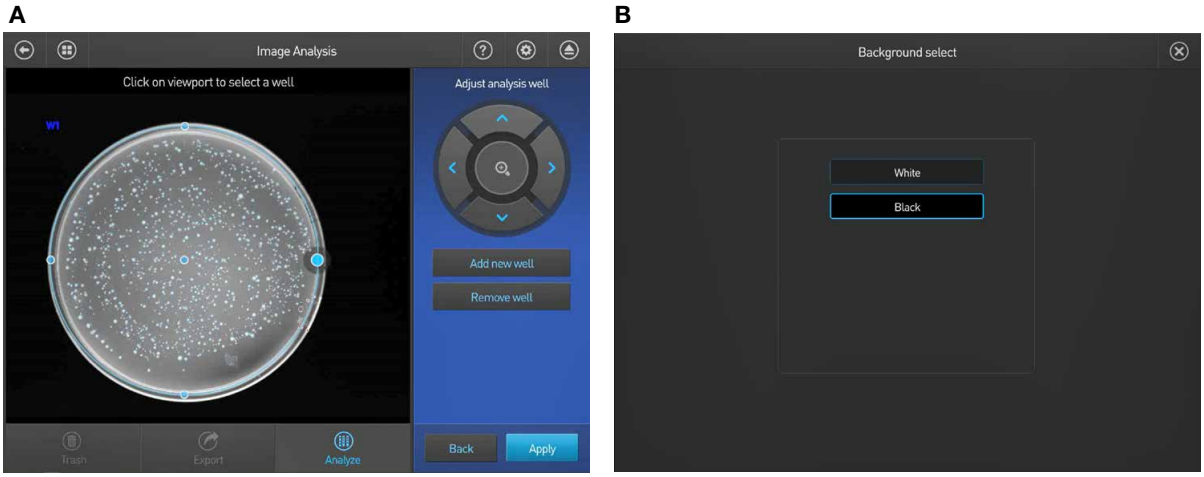


**Figure 9. Well adjustment (A) and background selection (B) screens.**

In the **More options** menu, use **Adjust colonies** and then select the colony designations desired for editing using the directional arrows to adjust the area specified around the colonies. Identified colonies can be removed by selecting **Remove colony** or added by pressing and holding the area where you want to add colony designations, followed by tapping **Apply** (Figure 11A, 11B). In **More options** (Figure 11A), select **Adjust image** to modify contrast, apply saturation, or invert the image (Figure 11C). Adjustments will be applied to individual channels.

In the **More options** menu, you can generate an analysis report by tapping **Generate report** (Figure 12A) and decide if you would like annotations, wells, or colonies to be displayed in the report by selecting the parameters you would like to be included. The report's file name can also be edited for export (Figure 12B). The report will contain the colony count analysis data and can be exported for documentation (Figure 12C).

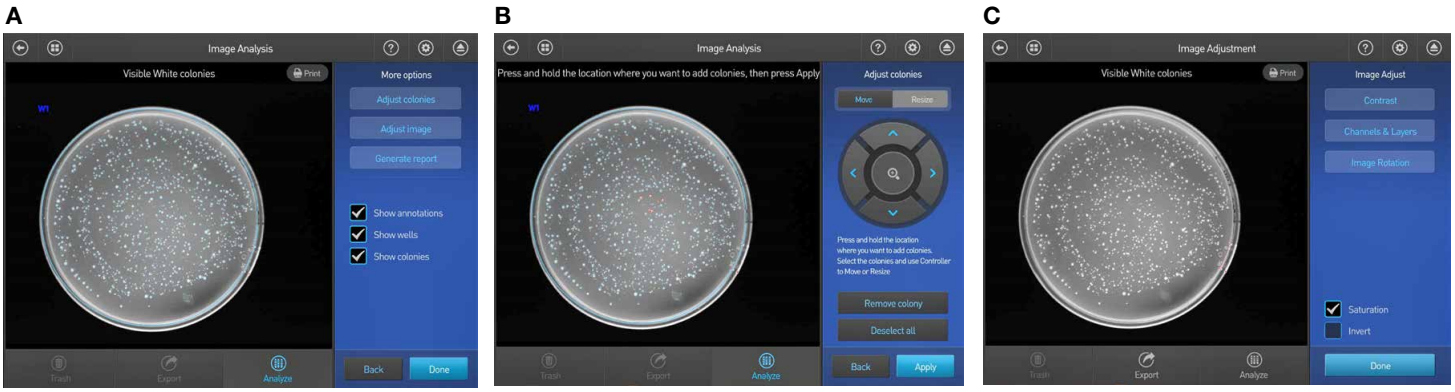


**Figure 11. The "More options" (A) and "Adjust colonies" (B) selections on the Image Analysis screen, and the Image Adjustment screen (C).**

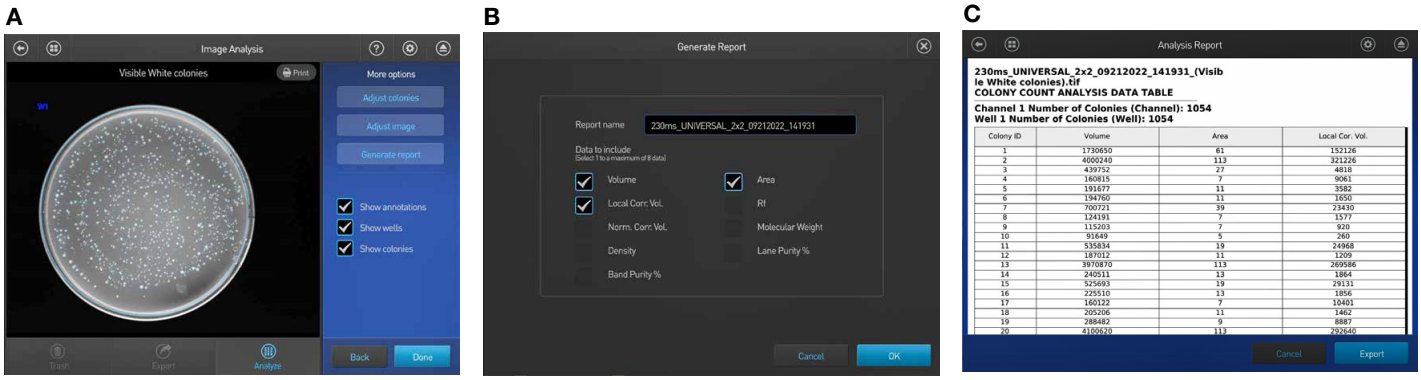


**Figure 12. An analysis report can be generated by selecting "Generate report" on the Image Analysis screen (A) and adjusting report parameters based on preferences (B); a final report can be exported (C).**

## Conclusions

The iBright Imaging Systems platform for western blot and gel documentation offers a streamlined approach to colony counting by enabling both imaging and analysis of colony plates directly on the instrument. Whether to perform a visual assessment or more in-depth analysis, our simple gating adjustments provide the flexibility required. In addition, the ability to generate a printable analysis report speeds up documentation of experiments.

## Ordering information

| Product | Cat. No. |
|---|---|
| **iBright FL1500 Imaging System** | |
| 1 instrument, including SmartStart Orientation and 2-year warranty | A44241 |
| 1 instrument, including SmartStart Orientation, 2-year warranty, and license for iBright SAE Software for 21 CFR Part 11 | A44241CFR |
| 1 instrument, including 1-year warranty | A44115 |
| **iBright CL1500 Imaging System** | |
| 1 instrument, including SmartStart Orientation and 2-year warranty | A44240 |
| 1 instrument, including SmartStart Orientation, 2-year warranty, and license for iBright SAE Software for 21 CFR Part 11 | A44240CFR |
| 1 instrument, including 1-year warranty | A44114 |
| **iBright CL750 Imaging System** | |
| 1 instrument, including digital SmartStart Orientation and 1-year warranty | A44116 |
| **iBright SAE Software for 21 CFR Part 11** | |
| 1 license (single license required per instrument) | A49208 |

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