RESHAPE

APPLICATION NOTE

Automated Halo Detection

For precise inhibition zone measurements

APPLICATION

Automating halos, or zone of inhibitions, with high-resolution imaging and AI

Why is it a critical need?

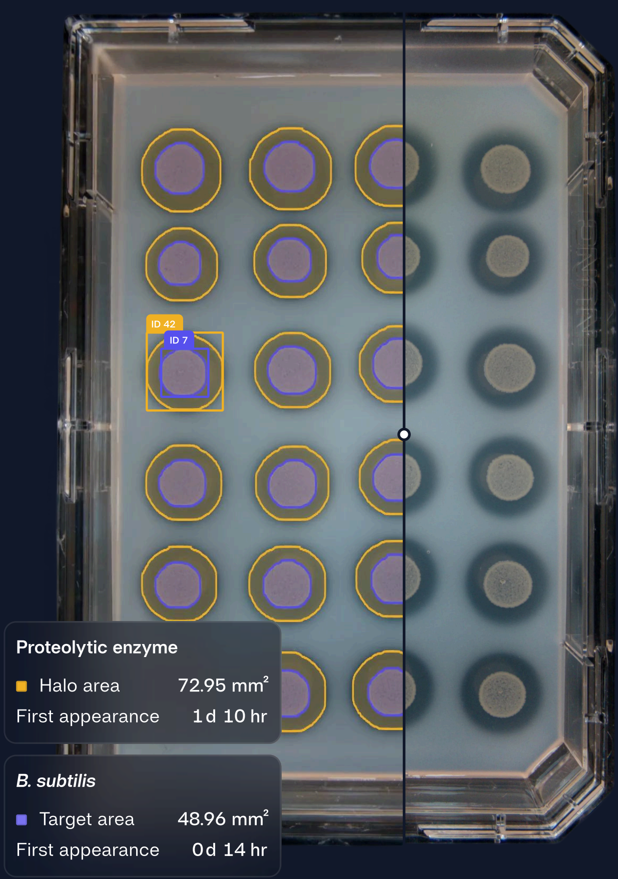
Accurate halo (zone of inhibition) measurement underpins antimicrobial susceptibility testing, enzyme screening, and microbial interaction studies. Manual measurements are slow, subjective, and difficult to scale, especially in large screening campaigns or non-standard plate formats. Endpoint-only readouts also miss early inhibition dynamics and lag phases. As labs move toward higher throughput and more data-driven decisions, they need reproducible, automated, and time-resolved halo quantification to ensure consistent results and deeper biological insight.

How does Reshape help?

Reshape combines controlled incubation, high-resolution time-lapse imaging, and AI-powered analysis to automatically detect and quantify halo zones across plate formats. The system continuously tracks halo emergence, growth kinetics, and endpoint diameters without user intervention, reducing variability and hands-on time. By enabling reproducible, scalable, and time-resolved measurements, Reshape transforms halo detection from a manual bottleneck into a standardized, high-throughput workflow.

Introduction

Halos, also referred to as zones of inhibition, are clear regions surrounding bacterial colonies or antimicrobial sources where microbial growth is suppressed. These zones can arise from secreted enzymes produced by bacterial cells that inhibit the growth of neighboring microorganisms, or from antimicrobial compounds, such as antibiotics, diffusing into the surrounding agar and preventing bacterial proliferation. In both cases, the result is a visibly clear area with no detectable growth. From here onward, these regions are referred to as 'halos' or 'halo zones'.



Accurate measurement of halo zones is a cornerstone of many microbiological assays, including antimicrobial susceptibility testing, enzyme activity screening, and microbial interaction studies. Traditional manual measurements are time-consuming and prone to user-to-user variability, particularly when applied to large screening campaigns or non-standard plate formats.

In this study, we explored automated, image-based measurement of halo zones across multiple plate formats, with the goal of ensuring accuracy, reproducibility, and compatibility with high-throughput screening workflows.

SHOWCASE STUDY

Antibiotic Disk Diffusion Assay

To evaluate the performance of automated halo zone detection, a standard antibiotic disk diffusion assay was performed and monitored over time using automated imaging and analysis.

Materials & methods

An *E. coli* culture was prepared and adjusted to three different inoculation densities (1×10^1, 1×10^2, and 1×10^3 CFU per plate equivalent). Each culture was mixed thoroughly with molten Mueller–Hinton agar and poured into plates to generate a uniform, confluent bacterial lawn.

Antibiotic disks were placed onto the agar surface using sterile forceps and gently pressed to ensure full contact with the agar. The following antibiotics were tested:

- Streptomycin (10 µg)
- Clarithromycin (30 µg)
- Penicillin (10 µg)

Plates were incubated at 30 °C for 20 hours in the Reshape Smart Incubator. Images were captured automatically once per hour to monitor the formation and expansion of halo zones over time.

Halo zone measurement

Zones of inhibition were quantified as the diameter of the complete clear halo zone surrounding each antibiotic disk. Measurements were reported in millimeters (mm) and calculated using automated image analysis, which detected the boundary between visible bacterial growth and the clear inhibition zone.

By acquiring time-resolved images throughout the incubation period, the system enabled continuous monitoring of halo development, allowing both endpoint and kinetic analysis of inhibition zone formation.

Results and discussion

Automated time-resolved imaging enabled continuous monitoring of antibiotic inhibition halo development across three bacterial inoculation densities (1×10^1, 1×10^2, and 1×10^3). Halo area was quantified over time for streptomycin (10 µg) and clarithromycin (30 µg), allowing both kinetic and endpoint comparisons (Figure 1).

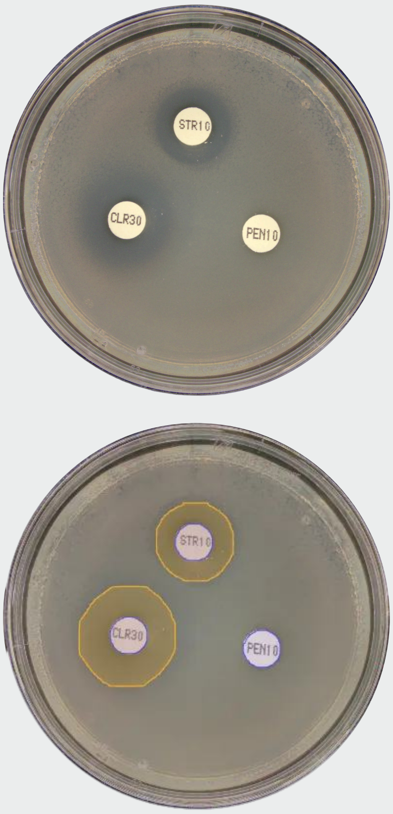


Figure 1A. Automated detection of the halo zone at 20 hours post incubation.

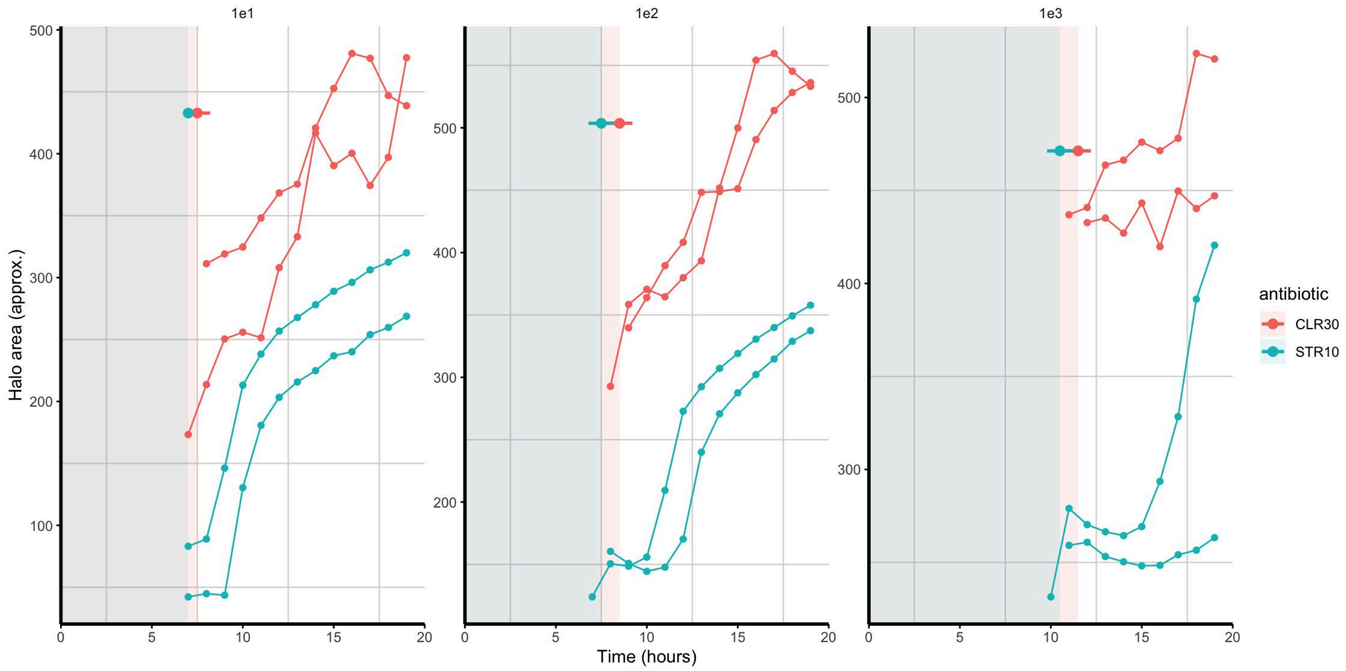


Figure 1B: Antibiotic inhibition (halo) zone development over 20 hours for clarithromycin (30 µg) and streptomycin (10 µg). Penicillin (10 µg) was also tested but produced no detectable halo zone and is therefore not shown. Data are shown for E. coli lawns prepared at 1×10^1, 1×10^2, and 1×10^3. The shaded region indicates the lag phase preceding the first visible halo. Markers represent the mean ± SD across replicate plates.

Halo emergence and lag phase

Across all inoculation densities, a distinct lag phase was observed prior to the appearance of a measurable halo zone. This lag corresponded to the time required for antibiotic diffusion and establishment of sufficient inhibitory concentration in the agar. The onset of detectable halo formation (defined as >10 area units) occurred earlier at lower bacterial densities and was delayed as inoculum density increased, consistent with higher biomass requiring greater inhibitory pressure.

The shaded regions in Figure 1B highlight this lag period, which was consistently captured by the automated analysis without user intervention. This demonstrates the system's sensitivity to early inhibition events that are often missed in endpoint-only assays.

Antibiotic-specific inhibition dynamics

Clear differences in inhibition dynamics were observed between the tested antibiotics. Clarithromycin (30 µg) produced larger and more rapidly expanding halo zones across all inoculation densities compared to streptomycin (10 µg). This difference was evident both in the rate of halo expansion and in the final halo area reached by the end of the incubation period.

Streptomycin exhibited slower initial halo expansion and greater variability, particularly at the highest inoculation density (1×10^3), where halo growth plateaued for several hours before increasing later in the incubation. This behavior highlights the value of kinetic measurements, as such transient effects would not be captured by a single endpoint readout

Effect of inoculum density

Increasing bacterial density resulted in delayed halo formation and reduced overall halo size for both antibiotics. At the lowest inoculation density (1×10^1), halos appeared earlier and expanded steadily over time. At higher densities (1×10^2 and 1×10^3), halo formation was delayed and growth rates were reduced, reflecting increased competition between bacterial growth and antibiotic diffusion.

Despite these differences, automated halo detection remained robust across all conditions, accurately tracking halo development even when inhibition zones were smaller or slower to emerge.

Reproducibility and automated measurement performance

Top markers in Figure 1B represent mean halo area ± standard deviation across replicate plates. Low variability between replicates demonstrates high reproducibility of both the biological assay and the automated image analysis pipeline. By eliminating manual measurements, the approach reduces user bias and enables consistent comparison across time points, antibiotics, and inoculation conditions.

Conclusion

This study demonstrates that automated imaging and analysis enables reliable, reproducible measurement of halo zones in antibiotic disk diffusion assays. By supporting continuous monitoring and high-throughput workflows, automated halo detection provides a scalable alternative to manual measurements for antimicrobial testing, enzyme screening, and microbial interaction studies.

The combination of controlled incubation, time-resolved imaging, and automated analysis makes this approach particularly well-suited for modern R&D laboratories seeking higher data quality with reduced hands-on time.