



LemnaTec SAW Bonit

HIGH THROUGHPUT AND HIGH-CONTENT SCREENING
OF CELLS AND CELL AGGREGATIONS IN 96 WELL PLATES

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Introduction

LemnaTec offers specific camera and lens systems, achieving the necessary resolution to quantify cells in 96 well plates. This high resolution imaging ranges down to one image per 96 well plate. The combination of advanced optics and powerful image processing makes quantification of cells and cell aggregations under high throughput conditions possible.

In combination with a plate transferring system, up to 100 plates can be read out without any human interference.

The results allow distinguishing of cells by size, colour and shape to provide high-content results for efficient sample characterisation.

Image acquisition

Industry standard high quality Sony 1.3 mega pixel cameras are used for image acquisition. Either colour cameras or similar black/white cameras with higher photosensitivity can be used, depending on the purpose of the analysis and on imaging conditions.

Application of highly developed macro zoom optics, which guarantee a depth of sharpness much higher than that ever achievable with single shot microscopes, permits sharp images, even if the agar in the cells has some variation in depth or the cells have spread into different layers of the agar.

Visible light (VIS) Illumination

To acquire images under visible light conditions, backlight and toplight is available. Dark field illumination can be used to enhance contrasts without staining the cells.

Fluorescence imaging

For specific fluorescence imaging, a wide range of different illumination and filter combinations is at hand. This includes high-intensity LED toplights that may be flashed to maximise intensity and minimise image acquisition time. Sets for GFP and other applications are of course available.

Resolution

With macro zoom optics between 1 and 6, wells can be imaged simultaneously. This leads to a technical resolution of 9 (1 well) to 22 μm (6 wells) per pixel.

For reliable object recognition, objects with a diameter of 2 to 3 pixels are necessary, depending on the contrast to the background and on background noise.

As mammal cells range exactly in size from 10 to 30 μm , use of the LemnaTec system allows recognition of many single cell assays and thus of cell proliferation assays as well. Determined by contrast, dark field illumination or specific cell staining can enhance results.

Image processing

For all image processing, the highly flexible and powerful image processing tools of all LemnaTec HTS systems can be used. This allows recognition of low contrast objects as well as separation of attached cells/cell aggregates. Artefacts or non-cell objects are eliminated automatically, based on criteria such as shape, size and colour.

The following example shows a cell-based stained assay.

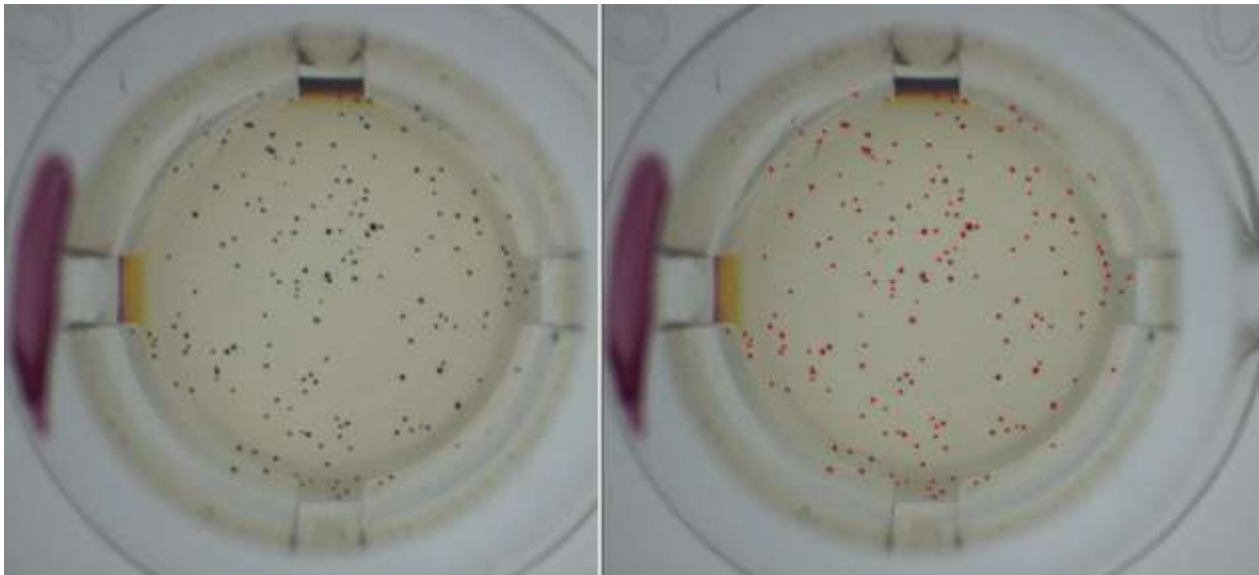
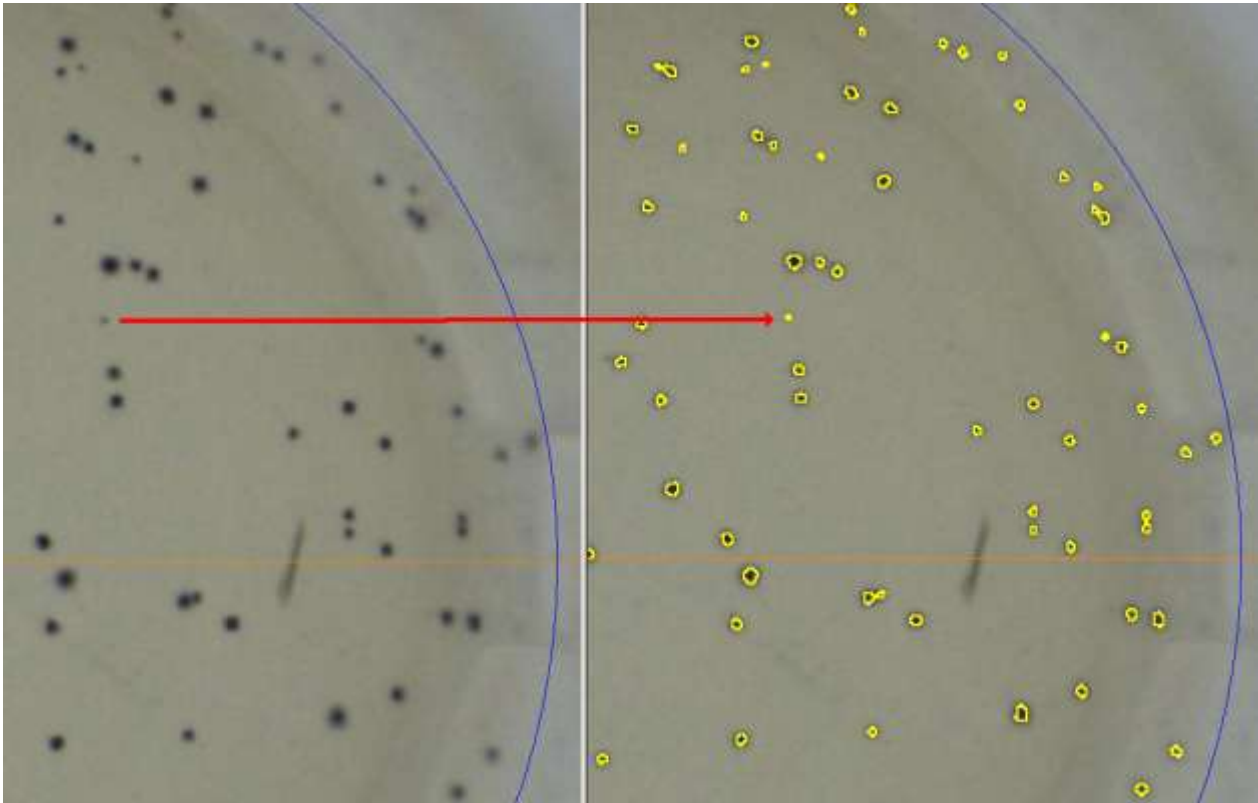


Image of stained cell assay. Images are taken in the single well per image mode. On the left side is the original image, on the right side the red rims around objects show recognised objects. Objects are identified automatically even under the difficult conditions near the rim of the well. High depth of sharpness compensates for the meniscus of agar which is therefore not seen in the image

The following image shows a magnified part of an original image and analysis. Each pixel represents $10\ \mu\text{m}$ at the magnification used.

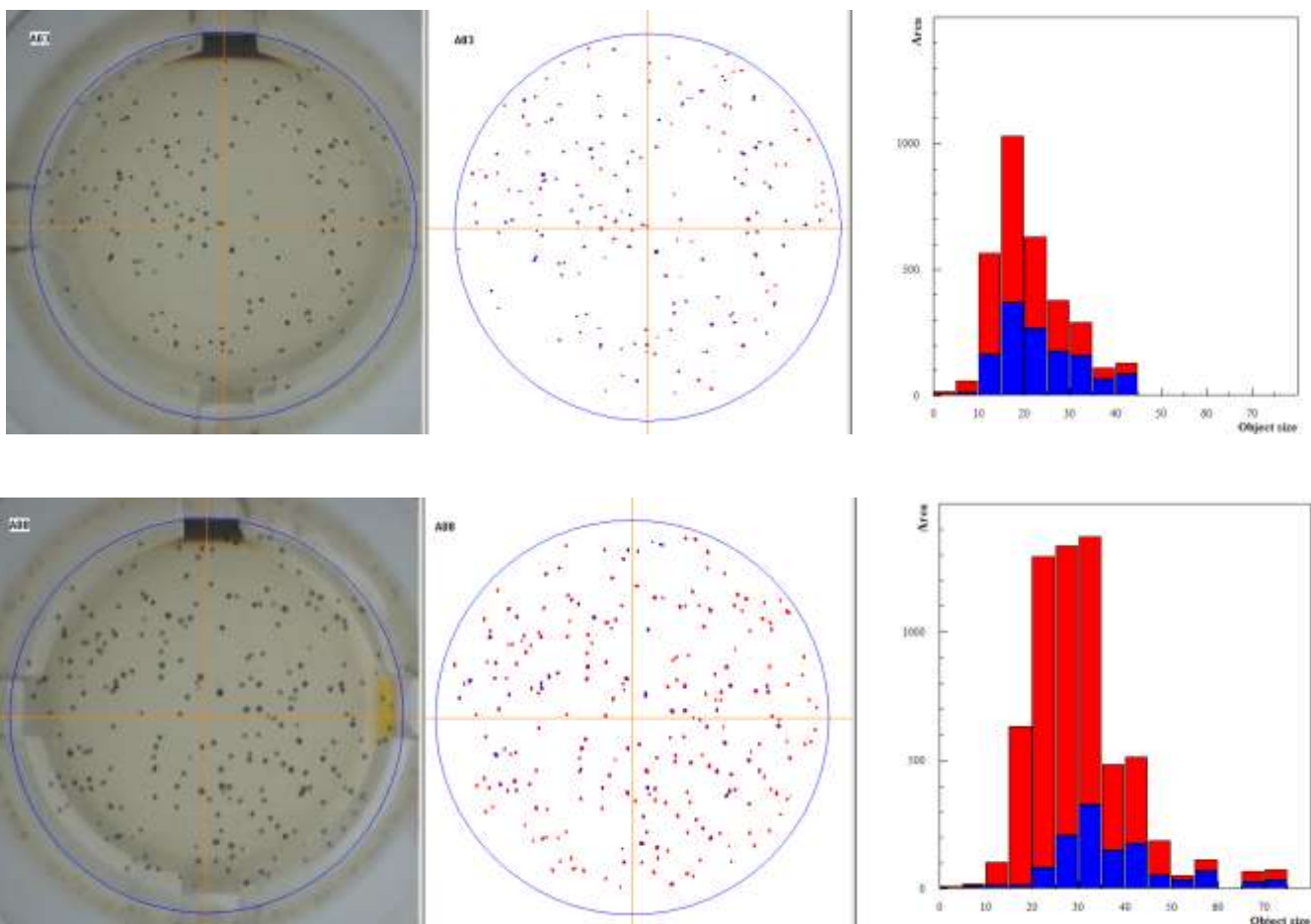


Magnification of a 96 well plate single well. The colony linked between the original image (left side) and the analysed image (right side) has a diameter of 3 pixels, $30\ \mu\text{m}$ respectively. Object separation and elimination of non-cell objects are done automatically

As fluorescent assays made with high flash impulses for excitation have a minimum of background noise, recognition of small objects is often even better than on VIS images, as displayed.

Results

Besides number, factors such as colour, colour intensity and total area of all cell clusters are quantified and stored in the database. Additional information like shape parameters, average colony size or size distributions can be made available for further data mining and analysis.



The two images show two different wells of a 96 well plate, containing 178 (upper) and 235 (lower) cell clusters. Average size and intensity of dye are significantly different for both treatments

The following table shows some of the key numbers that could be used to quantify and interpret differences between treatments in imaging results:

well	number of colonies	total area	av. size	% dark dye	% pale dye
upper	178	3190	17,9	39,8	60,2
lower	235	6236	26,5	18,6	81,4

Information in Databases

In the SQL-server database the following information is stored:

- ⇒ Original image, image with all objects, colour classified image
- ⇒ All results of the image processing as far as made quantitative (e. g. numbers, areas, colours)
- ⇒ Classification of hits based on the LemnaTec Hit Finder. This allows automatic classification of different effect types derived from quantitative measurements.
- ⇒ All image acquisition and image processing conditions (time, person, methods)
- ⇒ All information related to the well (plate number, well name, substance identification, etc.), as far as imported into the database (LIMS system)
- ⇒ Additional information like handwritten comments, finalisation of results for export

Allocation of the acquired data to the LIMS data (e. g. substance, concentration etc.) in the database is done automatically by a bar code identification and a bar code reader in the HTS Scanalyzer System.

The database is completely searchable without specific database application knowledge. PHP-modules for convenient database use from every computer linked to the server can be provided.

All information necessary within the database can be imported and later exported directly via SQL-protocols or data files to other electronic systems and file formats (e. g. *.xls, *.txt, *.html etc.)

Conclusion

The LemnaTec Scanalyzer provides fast and efficient cell screening capabilities in 96 well plates. Camera, lens systems and image processing allow to capture a wide range of data to comprehensively characterise the growth or behaviour of cells.

Combination of VIS and fluorescence imaging makes the Scanalyzer highly flexible in a wide area of different test assays.



For specific details on how to implement your cell screen or any further information, please do not hesitate to contact LemnaTec.

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