



LemnaTec SAW Bonit

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

Matthias Eberius
LemnaTec GmbH
Schumanstr. 18
52146 Würselen
Germany
+ 49 2405 4126-15
eberius@lemnatec.de
www.lemnatec.de

Introduction

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

In combination with a plate transferring systems up to 100 plates can be read out without human interference needed.

The results allow to characterise the cells by size colour and shape to provide high content results for efficient sample characterisation.

Image acquisition

For image acquisition industrial standard high quality Sony 1.3 Megapixel cameras are used. Depending on the aim of analysis and imaging conditions colour cameras or similar black/white cameras with higher light sensitivity can be used.

Use of highly developed Macrozoom optics to provide depth of sharpness much higher than ever achievable in single shot microscopes allow sharp images even if Agar in cells has some variation in depth or cells spread in different layers of the agar.

VIS illumination

To acquire image under visible light conditions fluorescent backlight and toplight is available. Dark field illumination is available to enhance contrasts without need of staining the cells.

Fluorescence imaging

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

Resolution

With the Macrozoom optics between 1 and 6 wells can be imaged simultaneously. This results in 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 contrast to background and background noise.

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

Image processing

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

The following example shows a cell based stained assay.

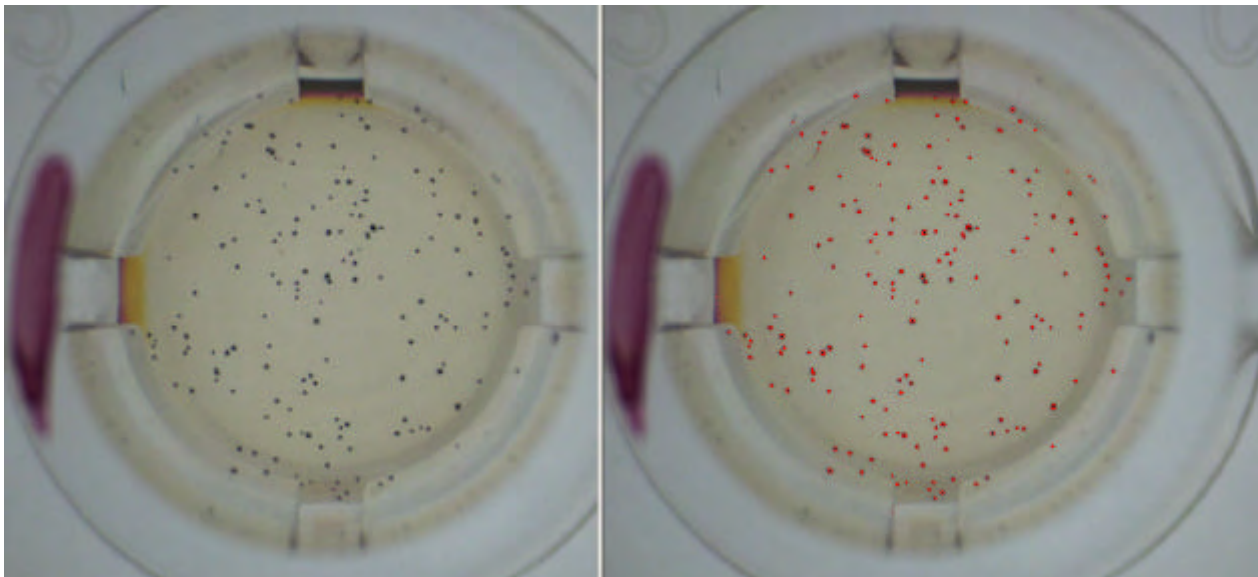
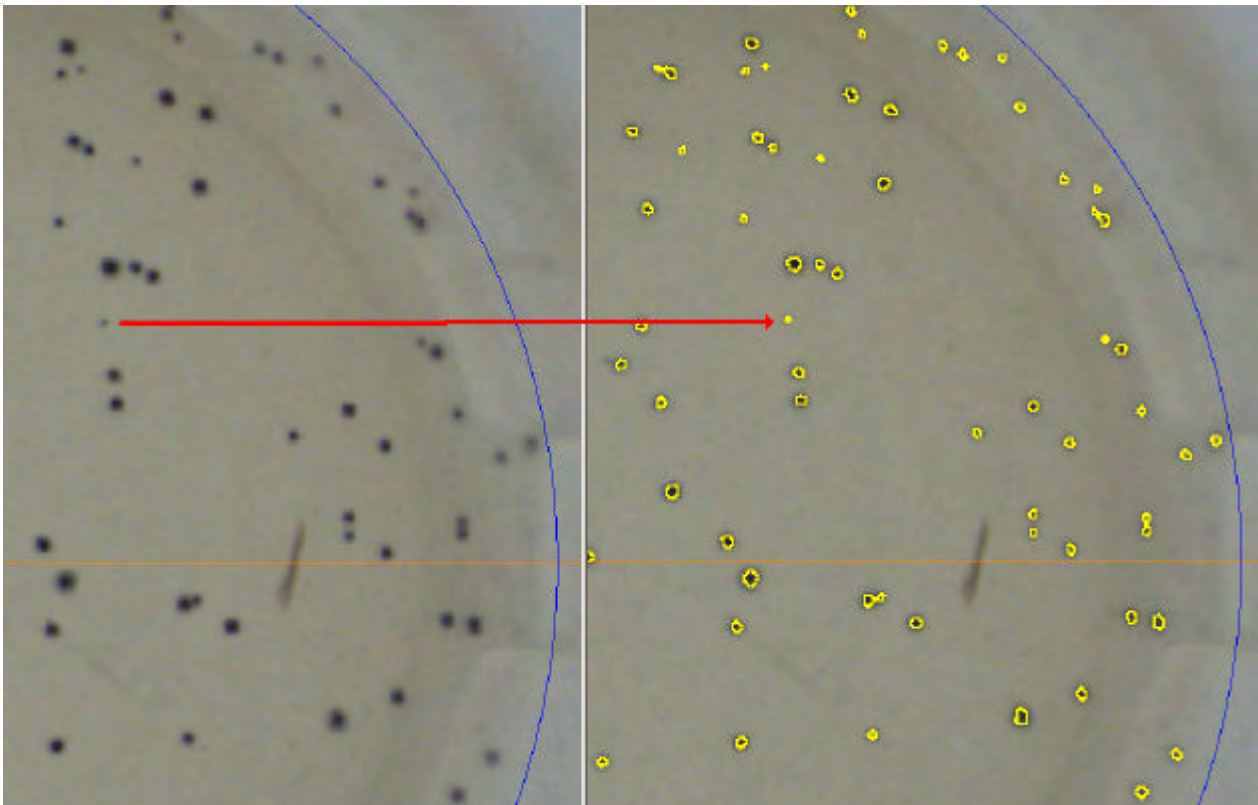


Image of stained cell assay. Images are taken in the single well per image mode. On the left side is the original image, on the right side the red rims of objects show recognised objects. Objects are identified automatically even under difficult conditions near the rim of the well. High depth of sharpness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

The following image shows a magnified part of one original image and analysis. Each pixel represents 10 μm at the magnification used.

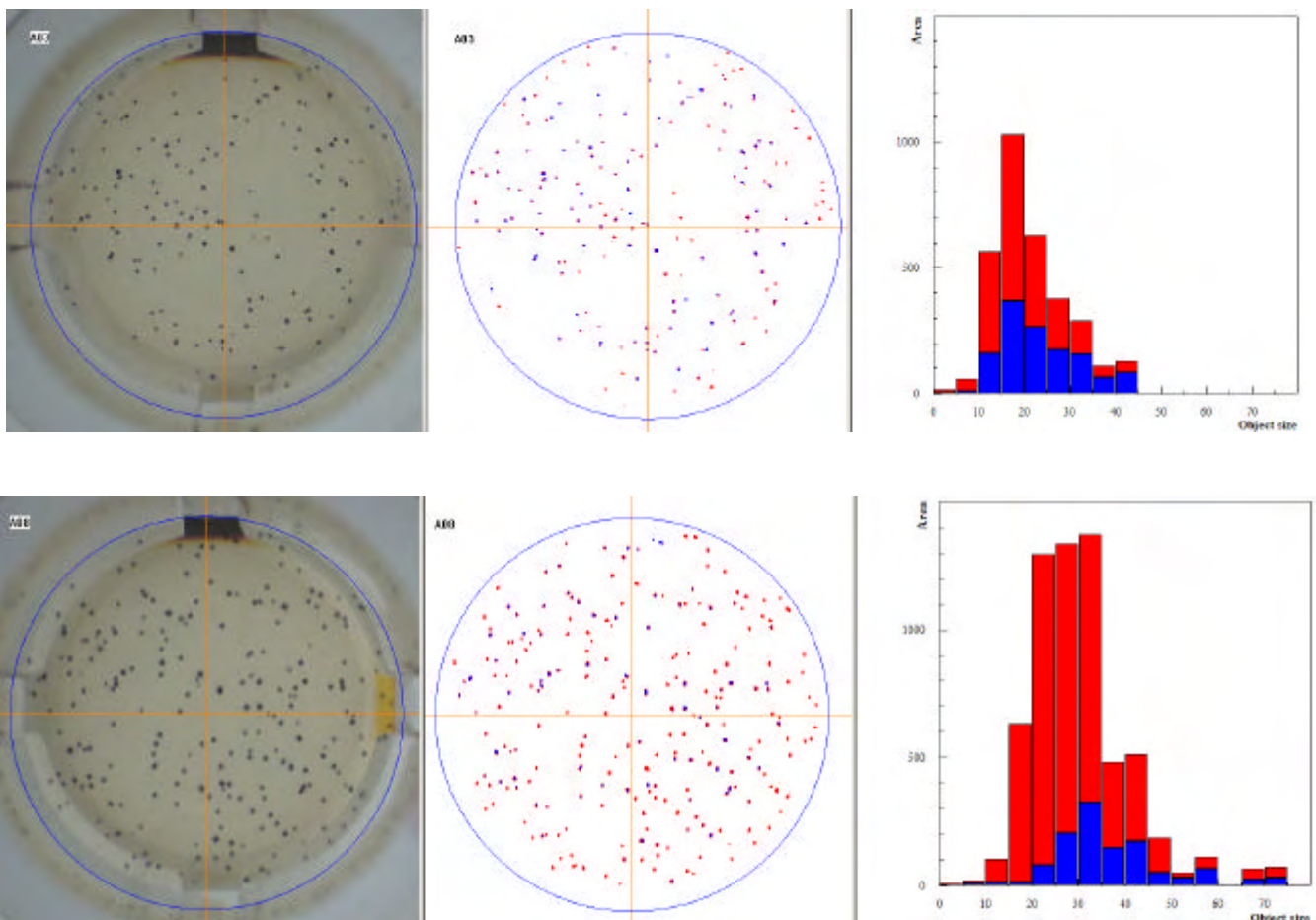


Magnification of a 96-well plate single well. The colony linked between the original image (left side) and the analysed image (right side) has a diameter of 3 pixels respectively 30 μm . Object separation and elimination of non-cell objects is done automatically.

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

Results

Besides number, colour/colour intensity and total area of all colonies is quantified and stored in the database, more 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 is significantly different for both treatments.

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

Well	Number 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

Data bases

In the SQL server-data base 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 types of effects 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 to the database (LIMS system).
- ? ?Additional information like manual comments, finalisation of results for export

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

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

All information necessary within the data base 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 characterise the growth or behaviour of the cells comprehensively.

Combination of VIS and Fluorescence imaging makes the Scanalyzer highly flexible for a wide range of different test assays.

For specific details how to implement your cell screen please do not hesitate to contact LemnaTec.