

High-throughput hybridoma-based antibody discovery using the Cell Metric™

Introduction

In the search for new drugs, the isolation of mAb-producing hybridomas targeted to a specific disease has long been the precursor to the mammalian cell line development process. This technique of producing hybridomas to secrete a specific mAb was first developed by Kohler and Milstein and they were recognised for their efforts with the Nobel Prize for the technology in 1984. Whilst there have been other competing methods in use, such as phage display technology, the hybridoma approach is still used globally in the production of therapeutic monoclonal antibodies and is having a resurgence. The drivers for improvement have been to find ways to make the process less manual, more efficient and also cost effective to implement.

This application note demonstrates how the Solentim Cell Metric™ imager can be used in the workflow to identify hybridoma colonies, monitor their growth and then be used for the sub-cloning process of the hybridoma cells.

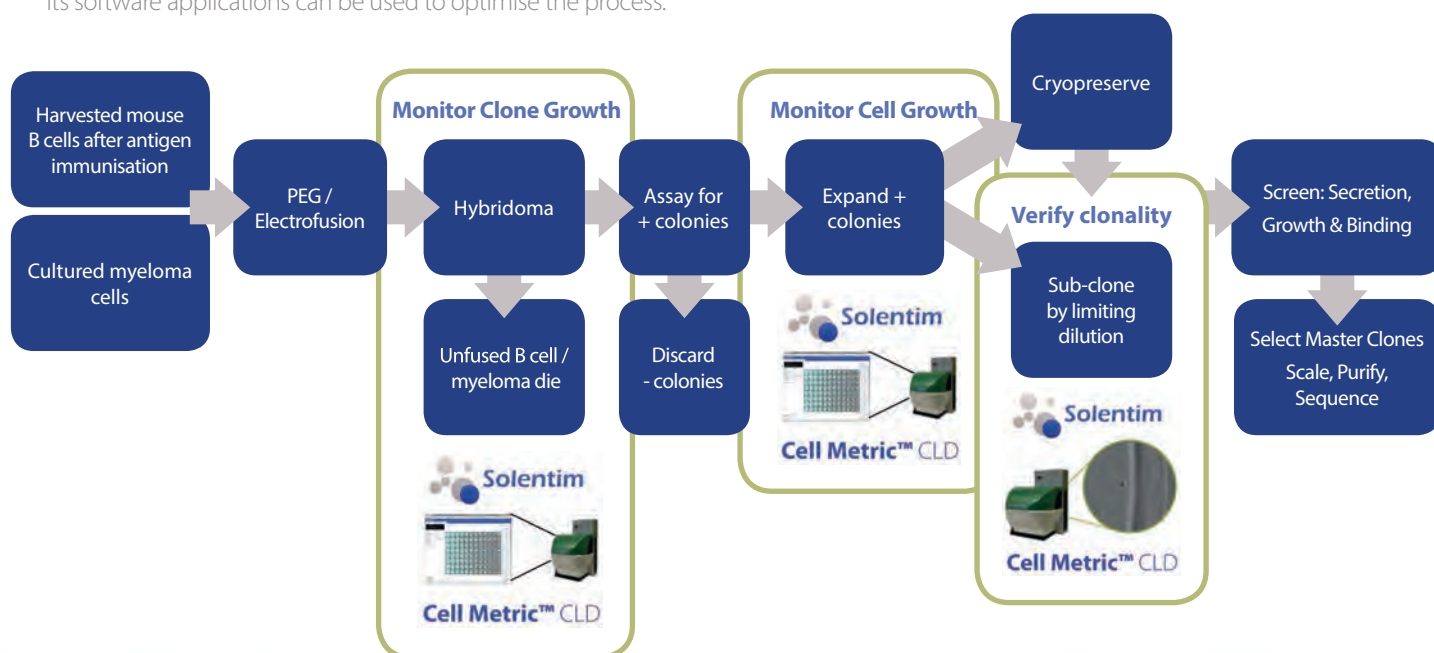
Methods

The workflow for production of hybridoma cells producing an antigen-specific mAb of choice can be a lengthy and multiple stage process (see **Figure 1**). The Solentim cell imaging systems (either Cell Metric or Cell Metric CLD) can be utilised in various ways throughout the process, offering the user multiple benefits:

- Eliminates the manual inspection of plates/wells using microscope
- Automates the measurement and tracking of colony formation, cell growth and confluence
- Documents the clonality of the positive cells

The system's multiple applications enable the user to repeatedly make use of the system's imaging capabilities to successfully select and process the hybridoma cells line through the entire development.

Figure 1: Workflow for the process by which Hybridoma cells, producing antigen specific mAbs, are generated and how the Solentim Cell Metric and its software applications can be used to optimise the process.



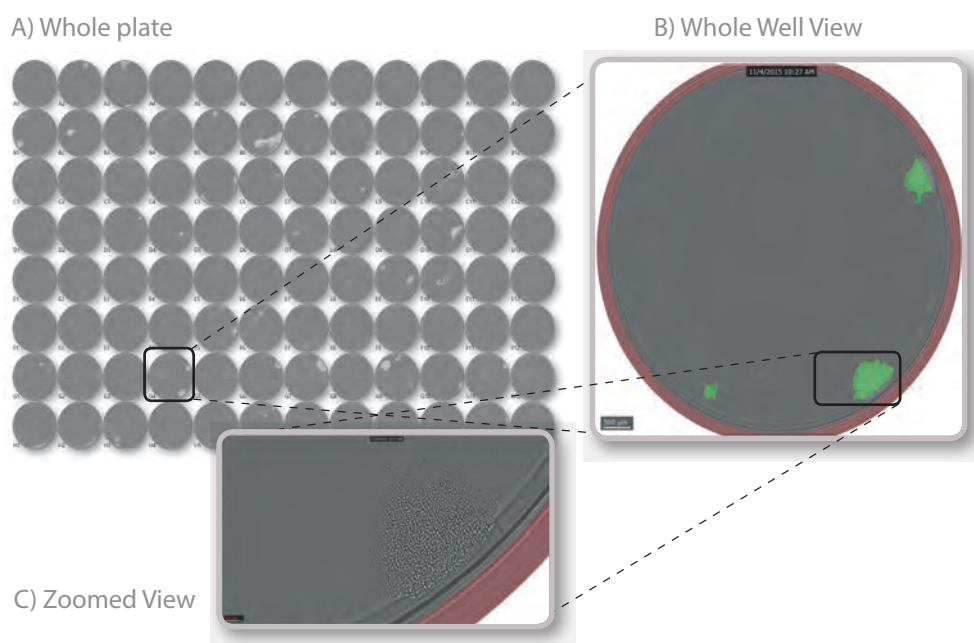
Step 1 - Monitor Colony Growth Application

After fusion, cells are seeded into 96 well (~200µl per well) or 384 wells plates and allowed to grow under selective pressure (hypoxanthine-aminopterin-thymidine – 'HAT') for between 7 - 21 days with no media changes. Plates are not moved during this period as the cells are loosely adherent. The plates are imaged during culture to monitor the cell growth and to identify colonies present (**Figure 2**). Wells are monitored and as the media turns

yellow (from pink), an aliquot is removed from the wells and assayed for antibody production and specificity. It should be noted that the Cell Metric is particularly good at resolving and identifying colonies against a background of dead B cells

This plate is not discarded but is continually monitored for any further, slower growing hybridomas that show good antibody production and specificity.

Figure 2: Images representing the capabilities of the Monitor Colony Growth application on the Cell Metric software. **A)** The thumbnail display of the well images captured for the entire 96 well plate. **B)** The display of well G4 showing 3 colonies that have formed in the well. **C)** Zoomed in image of one of the three colonies that have formed in the well.



Step 2 - Monitor Cell Growth and Confluence Application

Positive wells selected for expansion are transferred to 48 or 24 well plates (depending on how many positives hybridoma colonies are present). The Cell Metric is used again at this stage to capture whole well images of the cells under bright

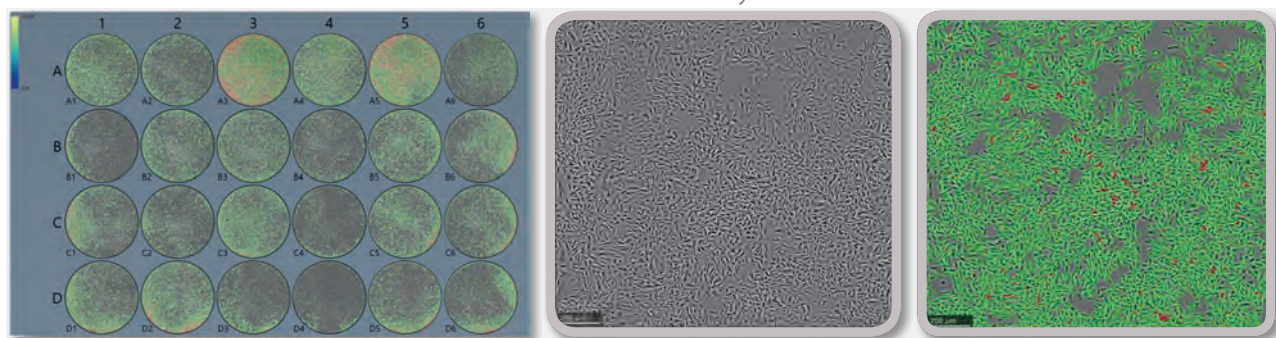
field conditions to monitor the cell growth and determine the confluence (**Figure 3**); once the wells reach exactly 80% confluence they are immediately cryopreserved so that they are not lost.

Figure 3: Images representing the capabilities of the Monitor Cell Growth application on the Cell Metric software. **A)** Whole plate, bright field image (thumbnail view) with software overlay on each well to measure confluence. **B)** Bright field image of highly confluent cells. **C)** Software overlay to measure confluence (monolayers are shown in false colour green; over-confluent regions are shown in red).

A) Whole plate, bright field image with confluence detection

B) Bright field image of confluent cell layer

C) Bright field image with confluent detection



Step 3 - Verify and Document Clonality Application

Limiting dilution (LD) cloning is carried in a 96 well plate using the best cryopreserved hybridomas. After seeding, images are captured as soon as the single cells have settled. The same plate is then subsequently imaged at regular intervals to capture the growth of the clones. Recommended time points for imaging are; day 0 (seeding time), day 1 (24 hours later), day 4/5 after seeding and then day 10-14 depending on the rate of growth.

Once all of the images are captured, the software compiles the images for interrogation to identify the wells containing the single cell-derived hybridomas (**Figure 4**).

As the clones grow, 5µl per well is screened again for antibody production, specificity and growth rate. The Specific Production Rate (SPR; the amount of IgG secreted per cell per hour) is calculated for each clone. Finally, using this information, the best clones are cryopreserved (for example, 1 master clone and 2 back-up clones) and the sequence/plasmid is passed onto the CHO cell line development group.

Evidence of the each of the single cell derived hybridoma cells produced is compiled in the software and documented for cell banking and the downstream CLD group (**Figure 5**).

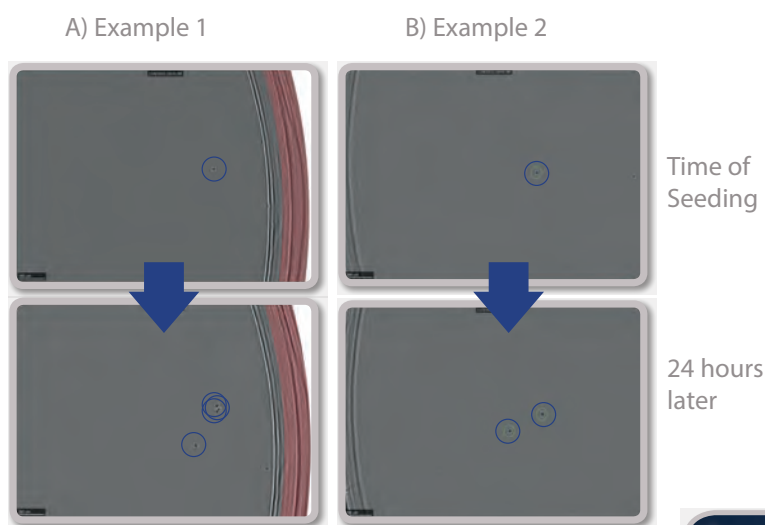


Figure 4:

Images representing the capabilities of the Verify Clonality application on the Cell Metric software. Two images were taken at time of seeding and 24 hours later.

A) Example 1, displays an image of a single cell captured at time of seeding that after 24 hours had divided into 4 cells and

B) Example 2, displays an image of a single cell captured at time of seeding that after 24 hours had divided into 2 cells.

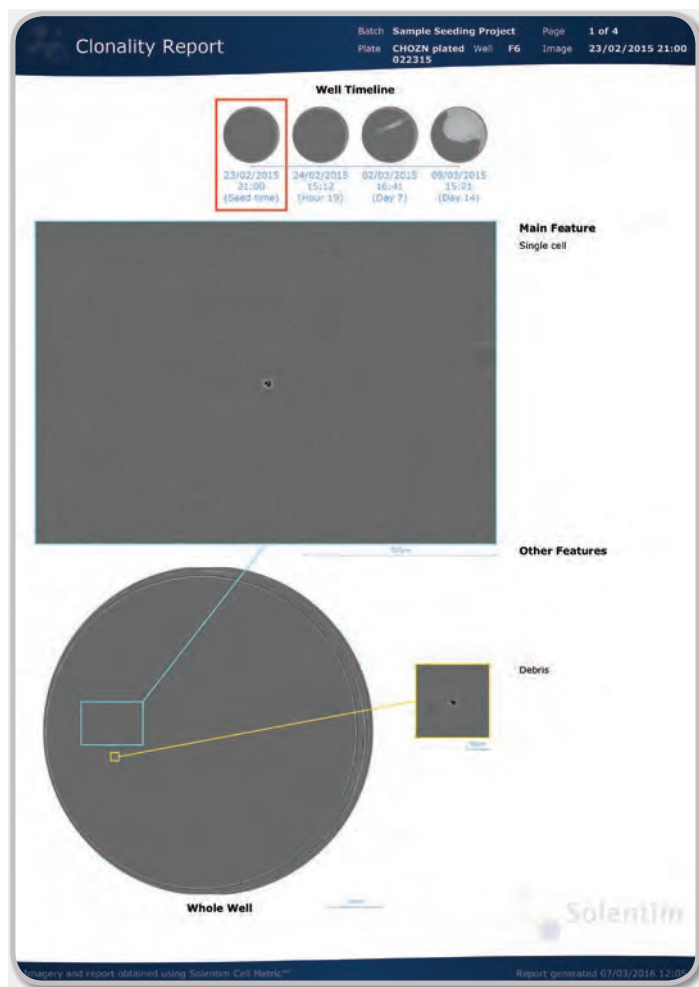


Figure 5:

Example clonality report generated by the Cell Metric software package. The report displays a whole well view and a zoomed view of the single cell present in the well of interest. The report is also able to highlight the single cell along with any artefacts present in the well as evidence of clonally derived cells.

Discussion and Conclusions

The image capture and multiple software application capabilities of the Cell Metric systems enable the user to use the instrument at multiple stages throughout the hybridoma workflow. This offers the following major benefits:

Speed and Efficiency - eliminates the manual inspection of plates/wells using microscope, allowing users to better use their time on multiple projects or other project activities.

Simple and cost effective to implement – Cell Metric automates multiple applications in one easy to use platform; identification of clones, monitoring cell growth and verifying clonality; eliminating the need for multiple instruments.

Improved objectivity - by producing accurate confluence data for consistent decision making (E.g. 80% confluence cut off for expansion/cryopreservation).

Automation – either using the incubated plate loader built in to the Cell Metric CLD or using the Cell Metric integrated with a third party robotic arm.

Provides traceability and documentary evidence - for internal downstream cell line development groups and cell banking, that the hybridoma cell developed is clonal.

Finally, as well as being used in conjunction with limiting dilution (as in this application) and FACS, the system can also be used in conjunction with the ClonePix system (picking from semi-solid media) for sub-cloning of the colonies picked.

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