Automated and Non-invasive Monitoring of Adherent Cell Cultures

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Abstract

Counting adherent cells is usually a destructive process since it implies detaching cells from their culture support. Cells can then be counted manually (Thoma or Burker chamber) or with an automated cell counter. In order to monitor adherent cultures over time, cells have to be seeded in separate vessels since samples are discarded/destroyed after counting.

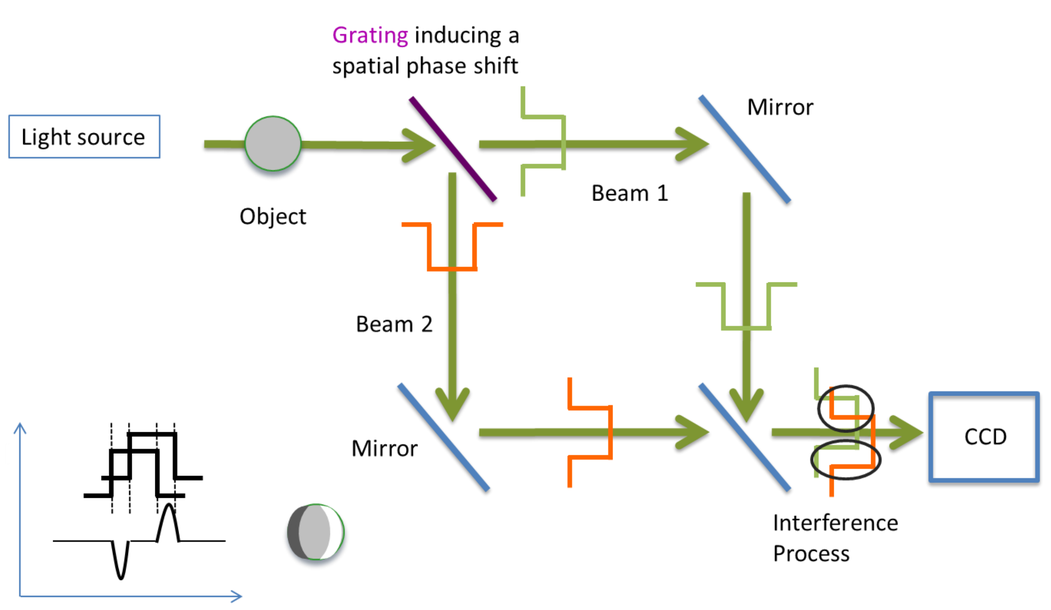
Monitoring adherent cells in culture vessels with **Differential Digital Holographic Microscopy** (DDHM) gives information not only on cell number and confluence but also on morphological parameters of the cells. DDHM captures information on both intensity and optical phase of the sample and combines them into a digital hologram. Thanks to the optical phase information, DDHM can quantitatively detect optical path changes (Quantitative phase contrast imaging) with a nanometric accuracy, allowing the detection of transparent objects and local composition changes.

The **iLine-M** version of the OVIZIO DDHM has been **designed for the monitoring of adherent cell cultures, from multi-well plates** for screening **to multi-layer vessels** for large scale cell production**. Screening is automated** and does not require sampling or staining. This technology circumvents the need for detaching cells for counting as it is the case with other cell counters. The very same sample can therefore be followed over time.

In this preliminary work, we compared the results generated by the iLine M with classical adherent cell counting methods which includes steps such as cell detachment using trypsin, and staining of the cells using Trypan-blue, before performing the actual cell count. Results indicate that the triplicate cell counts were reproducible over 3 days. The values were linear and fitted with the expected values, except for high cell densities, where they were slightly below expectations.

Working Principle

The technology is based on an improved Mach-Zehnder interferometer



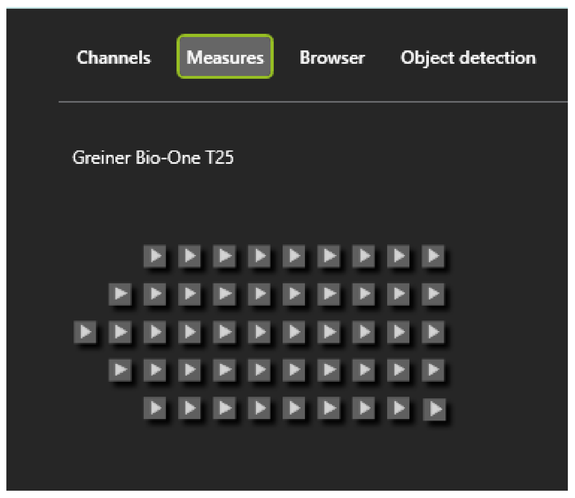
In Digital Holography, the scattered light beam from an illuminated object interferes with a reference beam on a CCD camera allowing for a 3D numerical reconstruction of the object. Differential Digital Holography is an evolution of this base technology that enables an important size reduction of the instruments and an increased stability (no frequent re-alignment of the beams required).

Advantages

- Non invasive, non destructive
- No detaching or staining required
- High reproducibility & repeatability
- Traceability at single cell level
- **Faster and objective** results in comparison to manual counting
- Monitoring of same sample over time
- Extraction of cell morphological data
- Ready for **automation**

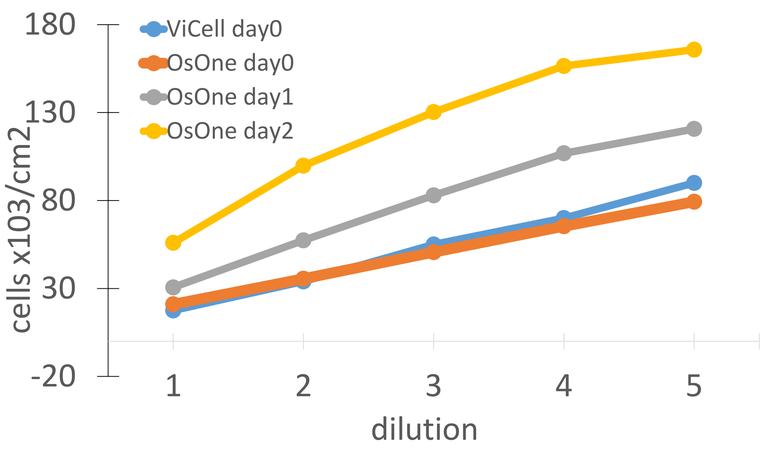
Materials & Methods

- iLine-M microscope (DDHM optical setup).
- **OsOne**, an all-in-one software, developed at OVIZIO, that controls the microscope, acquires and segments the images of the cells, and allows export of the results in various formats such as csv and fcs.
- L929 cells in DMEM (Lonza) + 10% FCS (GE Healthcare Life Sciences), cultivated in adherence on 48-well plates.
- Vi-Cell XR (Beckman-Coulter).

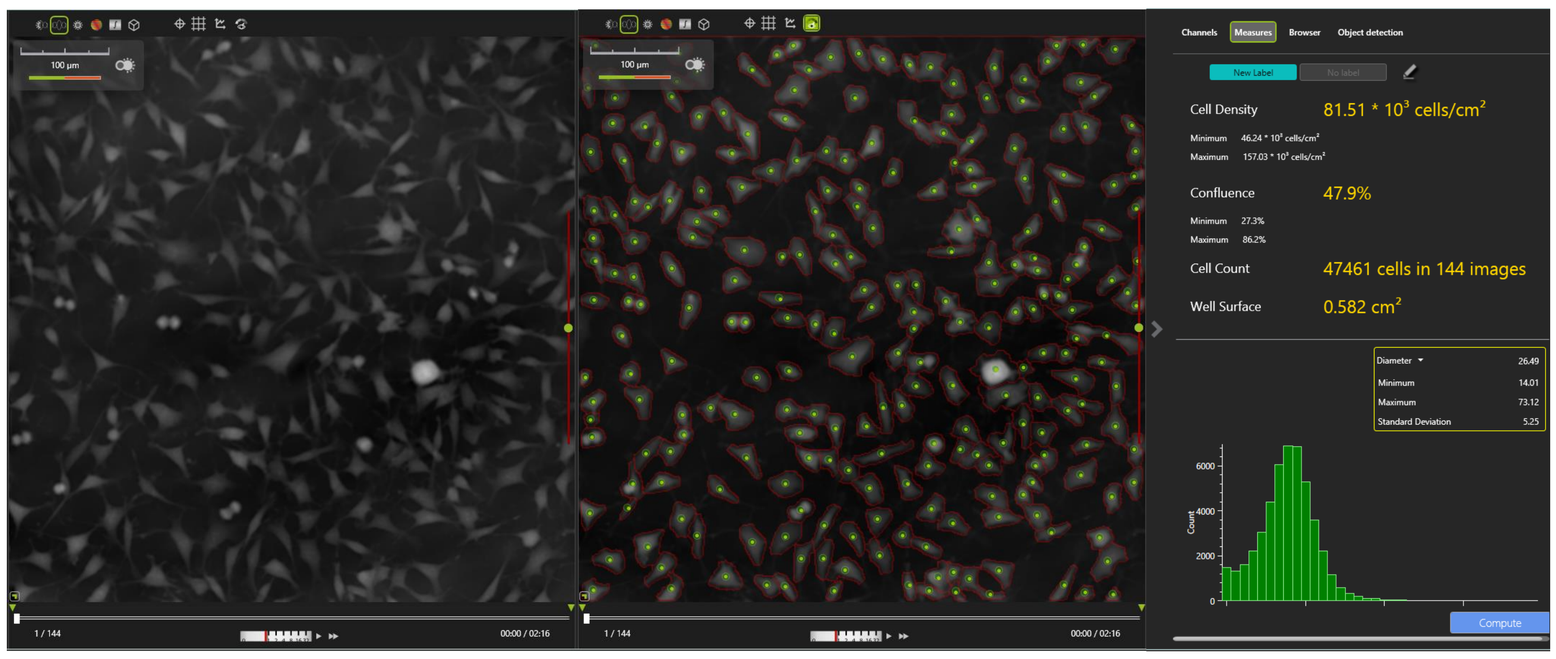


In OsOne, the user can select the areas of the vessel where captures are taken. Several types of vessels are pre-encoded in OsOne, from Petri dishes to T-flasks (various brands), up to multilayers vessels where captures and analysis are possible on several layers.

Results



Adherent cell count: L929 cells were diluted, seeded in triplicate in a 48-well plate and counted on 3 subsequent days. Results are shown for Vi-Cell count on day 0 and OsOne (averages of 3 counts) on days 0 (3:30 hours after seeding), 1 and 2. Analyses of the holograms are performed nearly in real-time.



Example of segmentation and adherent cell count for L929 cells cultivated in T-flasks

Conclusions

- Adherent cell count with OsOne is reproducible and convenient (automated, fast and easy to operate).
- Cell count on day 0 with OsOne matched values obtained with the Vi-Cell.
- Triplicate cell counts were reproducible for five dilutions over three days.
- Values were linear except for high cell densities.

Future Developments

- Complete the list of culture vessels available in OsOne.
- Complete selection of cell lines that can be analysed and segmented.
- One next step is to add temperature, humidity and CO_2 control so the sample can be incubated directly in the iLine-M.
- Furthermore, the DDHM technology is also capable to be used in combination with a fluorescence signal. OVIZIO's QMod module can be fixed on the C-mount (where the camera is usually attached) of a fluorescence microscope and transform it into a holographic microscope.

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