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Non-invasive and non-destructive measurements of confluence in cultured adherent cell lines

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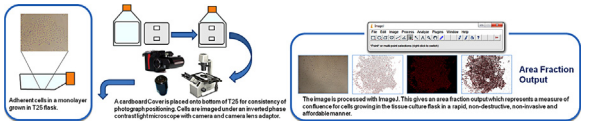
GRAPHICAL ABSTRACT



ABSTRACT

Many protocols used for measuring the growth of adherent monolayer cells *in vitro* are invasive, destructive and do not allow for the continued, undisturbed growth of cells within flasks. Protocols often use indirect methods for measuring proliferation. Microscopy techniques can analyse cell proliferation in a non-invasive or non-destructive manner but often use expensive equipment and software algorithms. In this method images of cells within flasks are captured by photographing under a standard inverted phase contract light microscope using a digital camera with a camera lens adaptor. Images are analysed for confluence using ImageJ freeware resulting in a measure of confluence known as an Area Fraction (AF) output. An example of the AF method in use on OVCAR8 and UPN251 cell lines is included.

- Measurements of confluence from growing adherent cell lines in cell culture flasks is obtained in a non-invasive, non-destructive, label-free manner.
- The technique is quick, affordable and eliminates sample manipulation.
- The technique provides an objective, consistent measure of when cells reach confluence and is highly correlated to manual counting with a haemocytometer. The average correlation co-efficient from a Spearman correlation ($n = 3$) was 0.99 ± 0.008 for OVCAR8 ($p = 0.01$) and 0.99 ± 0.01 for UPN251 ($p = 0.01$) cell lines.

ARTICLE INFO

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Method

This is a novel, non-invasive and non-destructive method developed to calculate a measure of confluence for growing adherent cells without disturbing growth. The result is known as an area fraction (AF) output which represents the amount of surface area that cells cover within a photographed area of a flask, representing an approximate value of confluence for the flask as a whole. This method can be used in order to monitor cells growing in T25 flasks or other cell culture plastics to determine objectively when they have reached confluence. This method can also be adapted for T75 flasks.

Recommended equipment

- ImageJ 1.45 s [1] (http://imagej.nih.gov/ij/download.html).
- Inverted Nikon TMS Phase Contrast Microscope (Nikon, Surrey, United Kindgom).
- Nikon Coolpix camera lens adapter (eBay Inc., US, seller-imaging_apparatus, United States).
- Nikon Coolpix 4500 camera (Nikon, Surrey, United Kingdom).
- Tissue Culture Flask T25, with red vented cap (Sarstedt, Wexford, Ireland).

Comparable products can be used for Microscope, camera, camera attachments and cell culture plastics. The cardboard cover slip used in this method (Fig. 1) is fashioned by measuring the flask dimensions and cutting cardboard into the correct shape using scissors.

Microscope preparation and photographing

1. The inverted Nikon (TMS) phase contrast light microscope is focused with the 4× objective lens in place.
2. For use with the Nikon Coolpix camera the Nikon Coolpix camera lens adapter is screwed onto the camera lens of the Nikon Coolpix 4500 camera. One of the eyepieces of the microscope is removed and stored safely. The camera lens adapter with attached camera is placed into the occular tube of the microscope. (When using other camera and microscope systems the camera is set up as per the cameras user manual.)
3. The flask of cells are taken from the incubator. The cardboard cover slip, designed to fit the bottom of the flask having 1 cm holes close to the top and bottom of the flask, is placed onto the bottom of the flask. See Fig. 1.

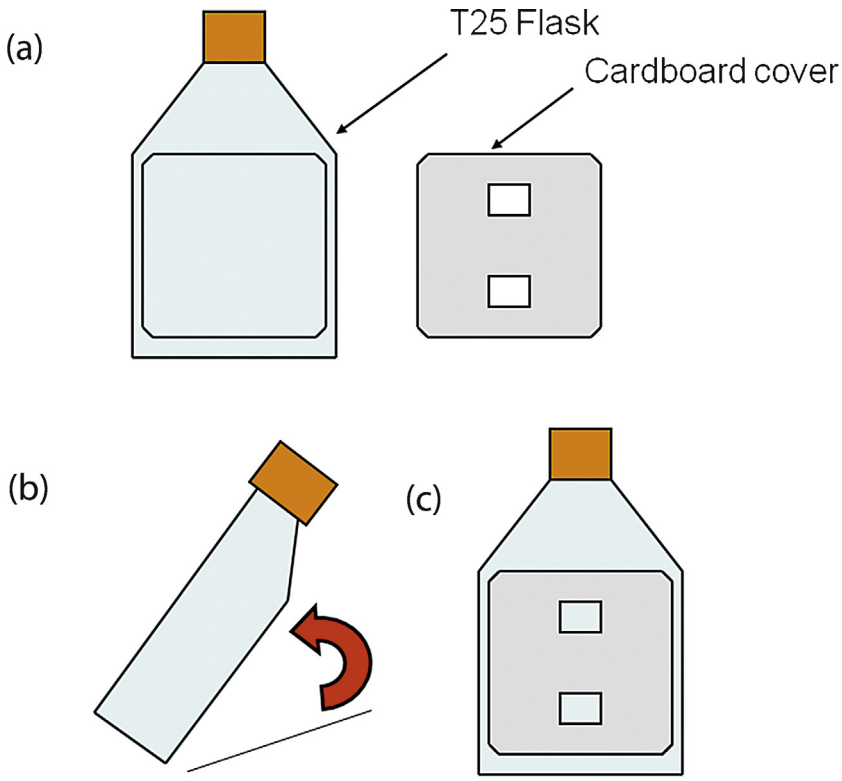

Fig. 1. T25 cardboard cover design for AF calculation method. (a) Shows the cardboard cover design next to T25 flask. (b) and (c) show how cover is placed onto a T25 flask before photography.