

speeding up angiogenesis assays using acumen[®] Cellista

introduction

Angiogenesis is a fundamental, multistep process by which new capillary blood vessels grow from pre-existing vasculature. It is of great interest in oncology research, as this process is critical for the progression of tumours from dormancy to malignancy. Each of the steps is highly controlled and regulated by angiogenic growth and survival factors that are secreted by the malignant cells as well as other cells within the tumor microenvironment. In pathological angiogenesis, however, these processes are not as well controlled and although the vascular initiation and formation stages occur, the vessels rarely mature, remodel, or regress in disease.

A better understanding of the exact mechanism of angiogenesis is required for the development of effective therapeutics, however, there are challenges to be met. Complex morphological assays such as angiogenic tube formation require large field of views to be analysed in order to gain robust, statistically significant data.

In a microscope-based imaging system, looking at a wider area can often result in very prolonged imaging times as their restricted field of view means multiple image captures are required for each well, and also for each colour. These images then need to be stitched together prior to actual data analysis, which also takes extra time.

acumen Cellista benefits

The acumen Cellista laser scanning imager offers rapid whole well image acquisition regardless of plate type (96- to 1,536-well plates). The acumen optics, where the field of view is 18 mm², allow the entire well area to be rapidly imaged and processed, with whole microplate read times as low as 5 minutes.

The acumen Cellista is also equipped with 405, 488, 561 or 640 nm laser excitation alongside the ability to simultaneously acquire up to four colours of fluorescence data per laser, allowing a wide range of fluorescent reagents to be detected in multicolour, multiplexed assays.

materials & methods

cell treatment

A 96-well microplate was pre-coated with matrix to induce tube formation. HUVEC cells were seeded out onto the microplate and incubated at 37°C for 18 hours. Cells were stained with calcein AM prior to analysis on the acumen Cellista.

Fluorescent signal was quantified using an acumen Cellista. This instrument is capable of simultaneously quantifying fluorescence from up to four different analytes. Immunofluorescent signals from the calcein AM staining were individually quantified on a per cell basis.

imaging

Whole well images across the plate can be captured in as little as 5 minutes per microplate. The acumen Cellista can generate open source TIFF files compatible with third party image analysis packages with no detriment to plate scan times to allow for secondary, more complex biology to be determined.



acumen Cellista. Whole wells are imaged on acumen as standard

key benefits

acumen Cellista provides the fastest way to perform multicolour cell-based imaging assays. It can:

- export open source TIFFs in as little as 5 minutes per microplate
- no image stitching on whole well images
- robust, statistically significant data

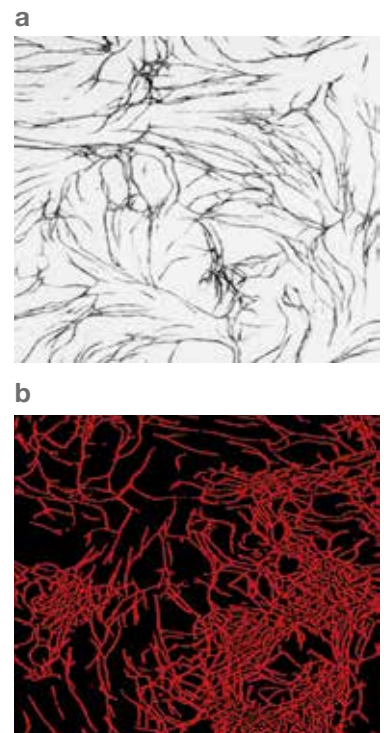


Fig 1. Well views imaged on an acumen. (a) Whole well open source TIFF image of tube formation in HUVEC cells stained with calcein AM. (b) processed image on an acumen Cellista showing classified angiogenic tubes based on size.

results

identification of VEGF-induced tube formation in HUVEC cells

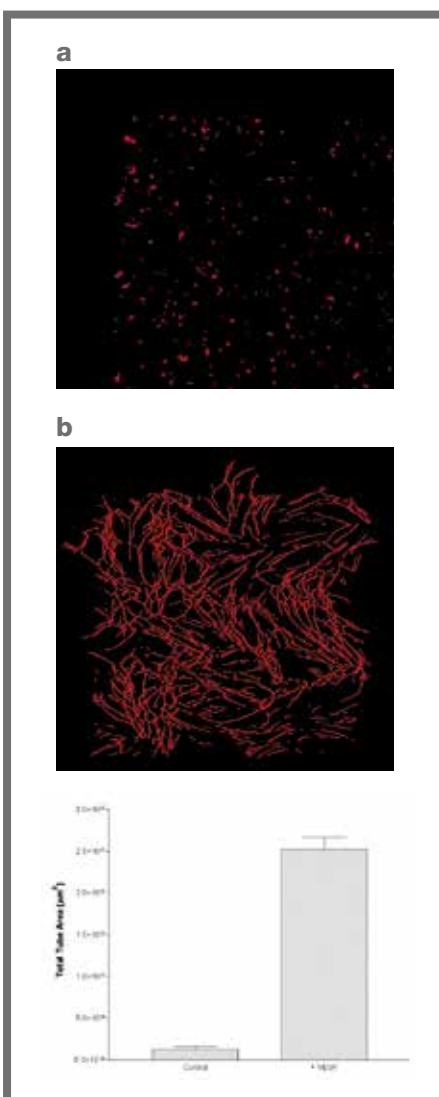


Fig 2. HUVEC cells stained with calcein AM and identified using size measurements. (a) Control well with no treatment. No tube formation was observed. (b) HUVEC cells treated with VEGF showing a large degree of tube formation. (c) Bar chart showing effect of VEGF treatment on total tube area.

open source TIFF files fit existing workflows

In addition to the in-built Cellista software which can simultaneously capture and process cell-based data, acumen Cellista can also export open source TIFF files, which can be processed in most free online and commercially available image analysis software packages. This extra functionality occurs concurrently with microplate processing, and therefore does not affect read times.

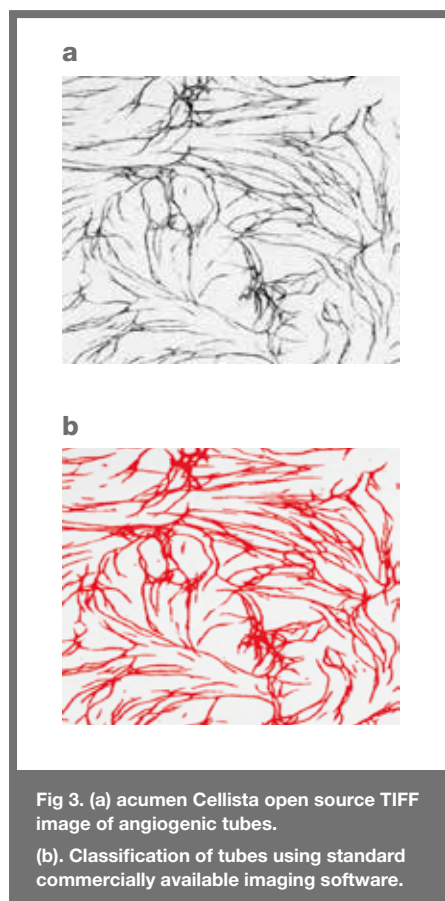


Fig 3. (a) acumen Cellista open source TIFF image of angiogenic tubes. (b). Classification of tubes using standard commercially available imaging software.

conclusion

This data demonstrates the ability of acumen Cellista to rapidly analyse and assess angiogenic tube formation in HUVEC cells. Since cell processing and labelling is performed with microplates, this technique is highly amenable to automation. The novel design features of acumen Cellista used in these studies permit whole well analysis at read times compatible with primary screening campaigns.

The benefit of this is that acumen Cellista can be used to quickly and easily export whole well TIFF files that seamlessly fit a company's existing image analysis process to determine more complex biology such as:

- total tube area
- total tube length
- number of tubes
- mean tube width
- total area of nodes
- number of nodes

acumen in publications

For further reading and employing acumen on angiogenesis assays see:

Isherwood., B. *International Drug Discovery*. 2011. Aug/Sep :34-37

Bowen & Wylie. *Assay and Drug Development Technologies*. 2006;4: 209-221

designed
for discovery



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