cellix

Cutting Edge Microfluidics

Mimicking *in-vivo* vascular and arterial flow

Cell rolling, adhesion and migration assays

cellix

Cellix Ltd., Unit 3.81,
Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's Street, Dublin 8, Ireland.

Tel: +353 (0)1 896 4016
Fax: +353 (0)1 896 2771
Email: info@cellixltd.com
URL: www.cellixltd.com

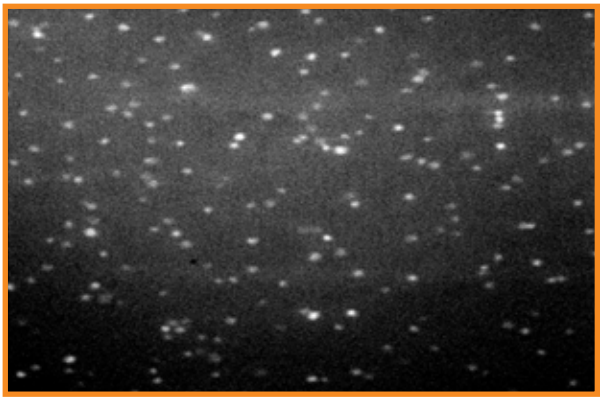
Shear Stress in the Human Body

Blood flows in veins, arteries, capillaries; saliva in the mouth, lung mucus inside the airways – all of these movements constantly create shear stresses that apply forces. Such forces can cause activation of cell surface receptors which lead to a very different picture compared to what is traditionally studied in static well plates.

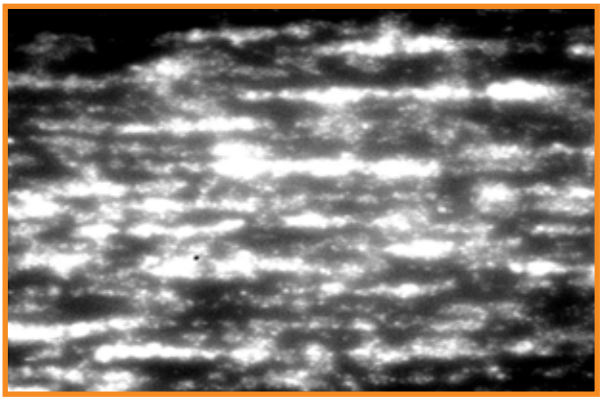
For example, vascular endothelial cells are exposed to fluid shear stresses that are typically 0.5 – 2 dyne/cm^2 on the venous side and 10 – 20 dyne/cm^2 on the arterial side of the circulation. Flow of saliva generates shear stress of 0.8 dyne/cm^2, applying drag to everything attached to the surface of teeth. Urine flow creates a shear stress of 0.17 dyne/cm^2 in the proximal renal tubule.

Cellix's VenaFlux™ Platform can accurately mimic shear stress / shear flow rates found *in vivo*. The VenaFlux™ measures cell adhesion to antibody-coated or endothelial-cell cultured microcapillaries, producing IC50 curves under shear stress conditions mimicking physiological flow. Data are more physiologically relevant resulting in a truly translational tool for drug discovery.

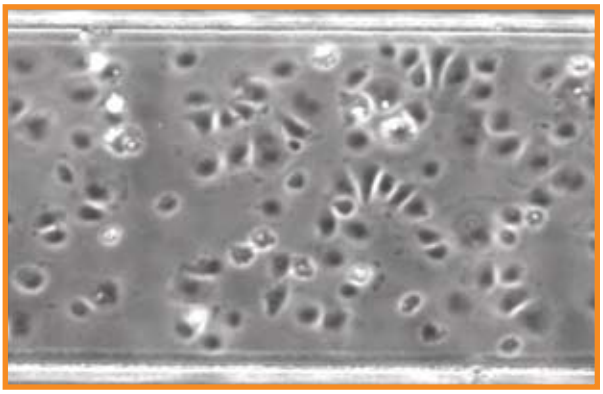
Difference between Static and Flow based adhesion assays



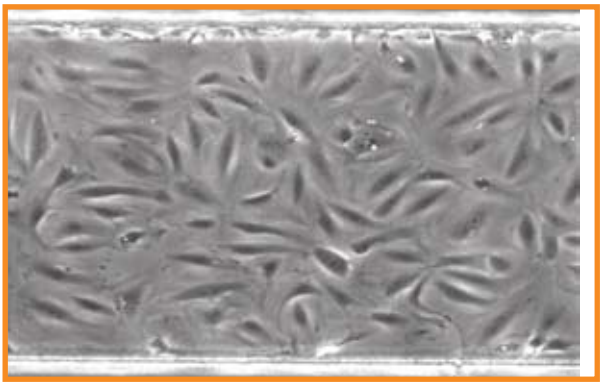
Platelet adhesion on vWF in static conditions



Platelet adhesion on vWF under flow conditions at a shear stress of 60 dyne/cm^2



Human endothelial cell culture in static conditions



Human endothelial cell culture under flow conditions at a shear stress of 10 dyne/cm^2

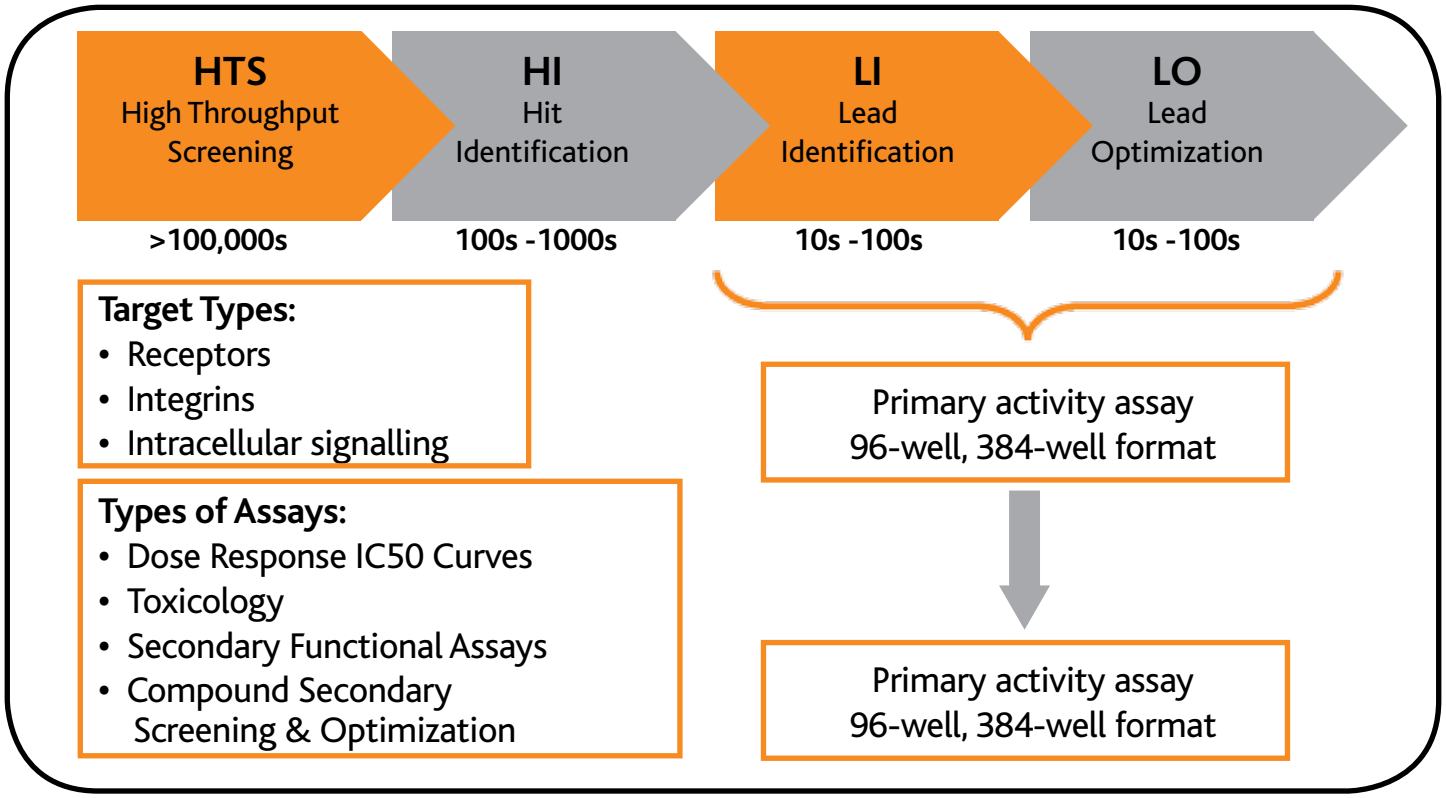
cellix

Cellix Ltd., Unit 3.81,
Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's Street, Dublin 8, Ireland.

Tel: +353 (0)1 896 4016
Fax: +353 (0)1 896 2771
Email: info@cellixltd.com
URL: www.cellixltd.com

Where is Cellix's Technology Used?

Flow Diagram of Drug Discovery Process uses Cellix's Platform:



Who is using Cellix's Technology?

Pharmaceutical & Biotech	Academic Institutions
AstraZeneca	University of Liverpool
BioTie Therapies	University of Reading
Sanofi-Aventis	National Institute of Health
Servier	Yale University

Why use Cellix's Technology?

Pharmaceutical & Biotech	Academic Institutions
Physiologically Relevant	Mimics *in-vivo* microenvironment.
Translational Research	Bridges the gap between static well plate assays and *in vivo* animal models.
Robust and Reliable	Technology is proven as a reliable screening tool in a vast array of therapeutic areas.
Faster and Cost Effective	Can analyse blood samples from animal models and pre-clinical cell-based assays in a matter of hours as opposed to months.
Flexible	Works with tiny (microlitre) samples of whole blood; wide range of cell suspensions and wide range of shear stress / shear flow rates.

cellix

Cellix Ltd., Unit 3.81,
Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's Street, Dublin 8, Ireland.

Tel: +353 (0)1 896 4016
Fax: +353 (0)1 896 2771
Email: info@cellixltd.com
URL: www.cellixltd.com

Therapeutic Areas – Cellix's Application Notes

Platform is fully validated for the following areas:

Atherosclerosis

Adhesion molecules, chemokines and oxidative stress in atherosclerosis.
Cellix Application Note: C300

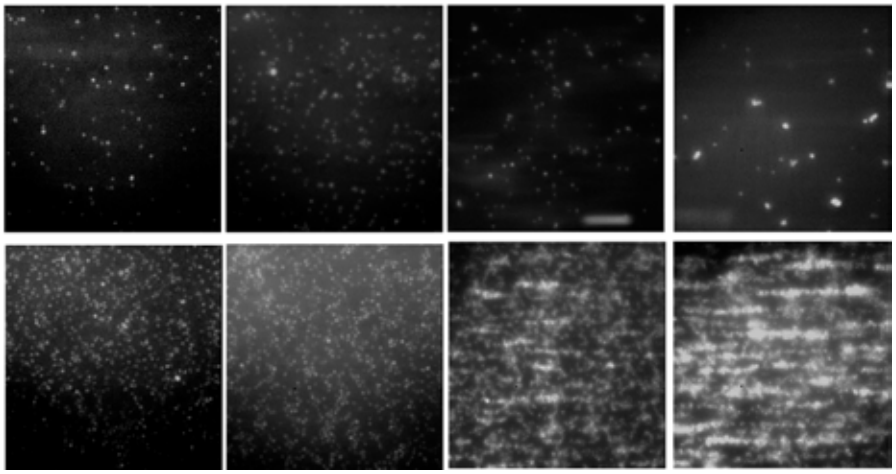
Control **oxLDL** **LDL+TNFα** **oxLDL+TNFα**

THP1 adhesion to endothelial cells under shear flow: Upregulation of adhesion molecule expression in endothelial cells due to oxLDL is much lower in comparison to TNFα effect.

Investigation into the role of different stimuli recognized to play an important part in atherosclerosis development (chemokines, cytokines, oxidized lipoproteins) on leukocyte adhesion to endothelial cells or purified adhesion molecules, under physiological flow conditions using Cellix's VenaFlux™ platform and biochips. Results from this study show Cellix's platform to be a robust tool to assess mechanisms involved in atherogenesis where **data were consistent with ApoE-/- mice models**.

Thrombosis

Mimicking Human Capillaries in an *in-vitro* set-up in relation to thrombus formation.
Cellix Application Note: C100



1 Sec

120 Sec

Flow Assay: Platelet adhesion from whole blood sample on vWF-coated Vena8™ biochips at time point 1 and 120 seconds; under shear flow rates of 4, 40, 60 and 120 dyne/cm^2.

Shear flow has a significant effect on activation of cell surface receptors. Platelets, in particular, show increased signs of adhesion corresponding to increased shear flow rates resulting in thrombi formation. Cellix's **VenaFlux™** platform & **Vena8™** biochips use microlitre whole blood samples to investigate the side effects of compounds on platelet adhesion, aggregation and thrombi formation.

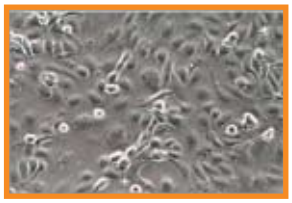
Immunology - Inflammation

Mimicking Human Capillaries in an *in-vitro* set-up for leukocyte adherence in inflammation.
Cellix Application Note: I200

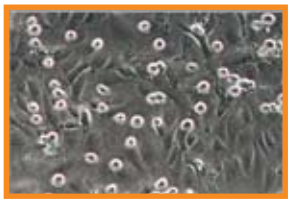
Cellix Ltd., Unit 3.81,
Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's Street, Dublin 8, Ireland.

Tel: +353 (0)1 896 4016
Fax: +353 (0)1 896 2771
Email: info@cellixltd.com
URL: www.cellixltd.com

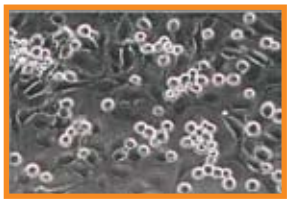
THP-1 adhesion to HUVEC



PMA-stimulated THP-1 adhesion to HUVEC



THP-1 adhesion to TNFα-stimulated HUVEC



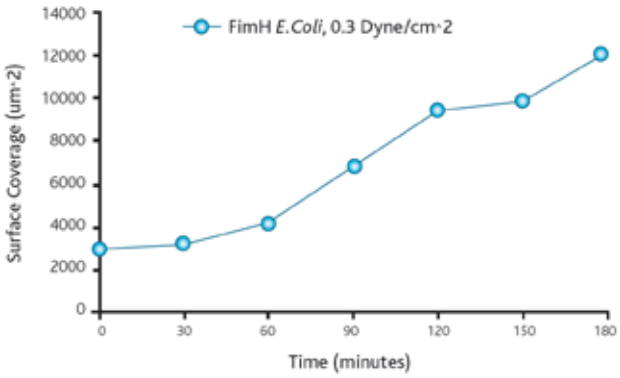
PMA-stimulated THP-1 adhesion to TNFα-stimulated HUVEC

This study presents data on the adhesion profiles of monocytes and PBMCs on a range of adhesion molecules and on endothelial cells. Analyzing the role of these proteins and other chemokines, which are involved in various stages of inflammation and leukocyte trafficking, under physiological flow conditions was achieved using Cellix's VenaFlux™ microfluidic platform.

Bacteriology

E. Coli adhesion and **biofilm colonisation** and formation on monomannase and trimannose-coated Vena8™ biochips.
Cellix Application Note: B100

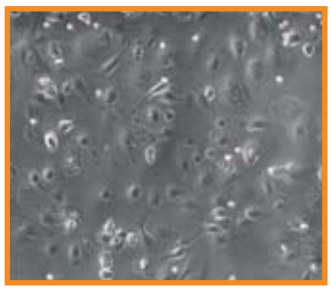
Type 1 fimbriae are the most common type of adhesive organelles in E. coli and mediate mannose-specific adhesion via the fimbrial tip-associated lectin-like subunit FimH. FimH mediates `catch-bonds' with mannose that are strengthened by tensile mechanical force. The environmental shear stress is of great importance and studies such as this enables researchers to gain an insight into the mechanism by which bacteria thrive, becoming resistant to therapies. Cellix's VenaFlux™ platform can accommodate extended periods (e.g. weeks) of sample perfusion of biofilm culture studies.



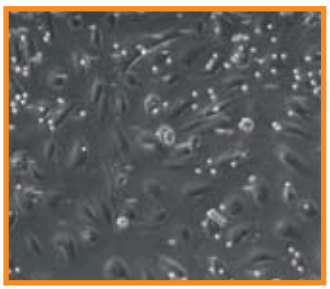
Time dependent formation of biofilm: E. coli was subjected to shear flow rate of 0.3 dyne/cm² for 3hrs on monomannose-coated Vena8™ biochips. Surface coverage of bacteria measured as µm².

Respiratory – Asthma and COPD

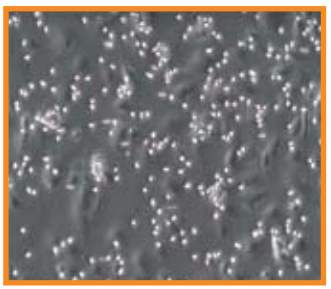
Mimicking Human Capillaries in an *in-vitro* set-up for leukocyte adherence in inflammation.
Cellix Application Note: R200



Eosinophil adhesion to HMVEC



Eosinophil adhesion to TNFα-stimulated HMVEC



Eotaxin-stimulated eosinophil adhesion to HMVEC

Eotaxin-stimulated eosinophil adhesion to TNFα-stimulated HMVEC

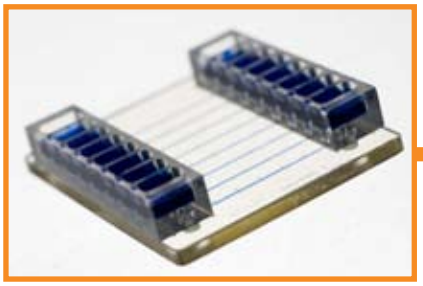
Cellix's VenaFlux™ platform and Vena8™ Biochips were used to determine novel anti-inflammatory effects of montelukast on resting and GM-CSF stimulated eosinophils. New drug targets were identified through eosinophil adhesion to endothelial cell markers.

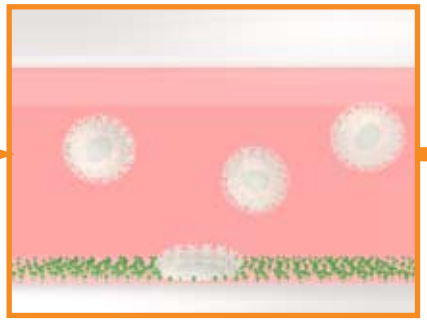
Cellix Ltd., Unit 3.81,
Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's Street, Dublin 8, Ireland.

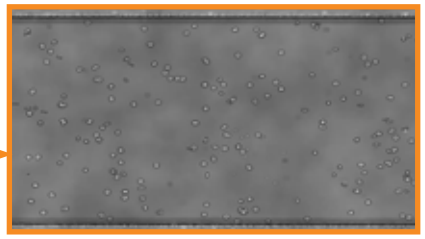
Tel: +353 (0)1 896 4016
Fax: +353 (0)1 896 2771
Email: info@cellixltd.com
URL: www.cellixltd.com

Cellix's Biochip Range

Vena8™ Biochip for cell-receptor ligand rolling, binding/adhesion assays under shear flow.





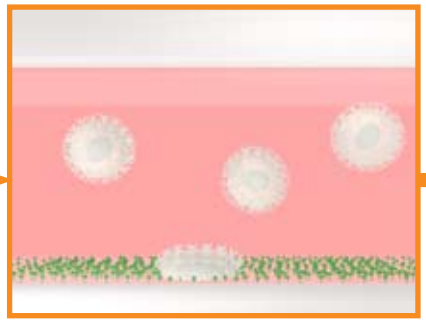


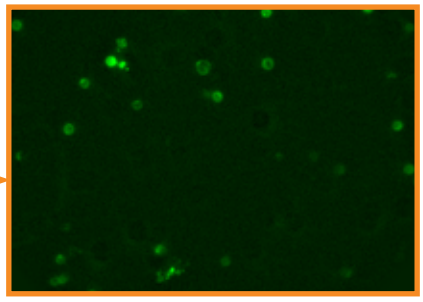
No. of channels	8
Substrate Thickness	0.5mm

Illustration: Cell adhesion inside ligand-coated microchannel of Vena8™ Biochip

Example: Adhesion of RBCs infected with malaria parasite to ICAM-1 coated channel at shear stress of 0.5 dyne/cm²

Vena8 Fluoro+™ Biochip for cell-receptor ligand rolling, binding/adhesion assays under shear flow. Ideal for fluorescent immunostaining or **confocal microscopy.**



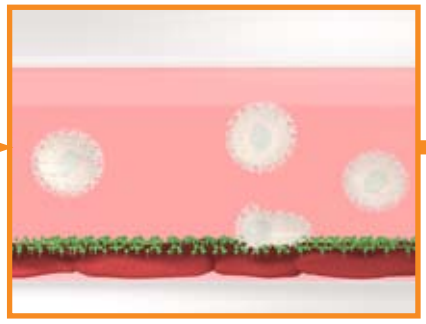


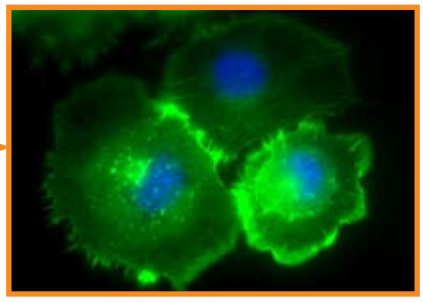
No. of channels	8
Substrate Thickness	0.17mm

Illustration: Cell adhesion inside ligand-coated microchannel of Vena8™ Biochip

Example: Adhesion of Human PBMC (stained with FITC conjugated to CD3 antibody) to VCAM-1 coated channel at shear stress of 0.5 dyne/cm²

Vena8 Endothelial+™ Biochip - COMING SOON! For cell-cell interaction (rolling, adhesion, migration) studies under shear flow. Endothelial cell monolayers easily cultured inside microcapillaries.





No. of channels	8
Substrate Thickness	0.5mm

Illustration: Cell-cell adhesion inside endothelial-cell cultured on microchannel of Vena8 Endothelial+™ Biochip

Example: Endothelial cell line staining in Vena8 Endothelial+™ biochips; Green staining - Phalloidin (filaments) Blue staining – Hoechst (nucleus)

Cellix Ltd., Unit 3.81,
Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's Street, Dublin 8, Ireland.

Tel: +353 (0)1 896 4016
Fax: +353 (0)1 896 2771
Email: info@cellixltd.com
URL: www.cellixltd.com

VenaEC™ Biochip for cell-cell interaction (rolling, adhesion, migration) studies under shear flow. Endothelial cell monolayers easily cultured on substrates which may be dis-assembled.

No. of channels	2
Substrate Thickness	1.0mm

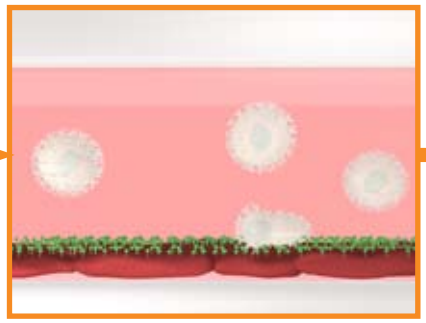
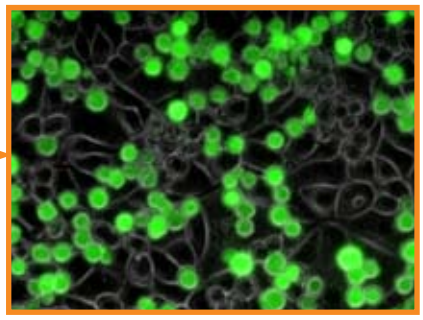


Illustration: Cell-cell adhesion inside endothelial-cell cultured on microchannel of VenaEC™ Biochip



Example: MDA-GFP adhesion on TNF treated HUVEC cell line at shear stress of 0.5 dyne/cm²

Biochips for investigating cell receptor-ligand rolling, binding/adhesion studies of cell-cell interaction (rolling, adhesion, migration) under conditions mimicking physiological flow. Each microcapillary may be coated with a different adhesion molecule. Cell suspensions are injected using the Mirus™ Nanopump which supports a range of shear flow rates. For applications of high shear flow rates (above 2 dyne/cm²) such as thrombosis; Cellix biochips are compatible with syringe pumps from other suppliers such as **Harvard Apparatus, World Precision Instruments, KD Scientific** and many more. For lower shear flow rates, it is advisable to use Cellix's precision syringe pump, the Mirus™ Nanopump. All Biochips are sold in packs of 10 facilitating 80 experiments per pack.

Biochip	Microscopy	Features	Applications
Vena8™	• Brightfield / phase contrast • 20X, 40X, 60X magnification	• Clear, transparent plastic • Grid marks for manual positioning • Coating with range of ligands: VCAM, ICAM, selectins, collagen, fibrinogen, vWF • Assay cell sample volume <10 uL per channel	• Cell rolling and adhesion studies • Thrombosis • Cell proliferation
Vena8 Fluoro+™	• Brightfield / phase contrast / fluorescence / **confocal** • High magnification: 60X, 100X • High numerical aperture • Low fluorescent background	• Improved optical clarity • Coating with range of ligands: VCAM, ICAM, selectins, collagen, fibrinogen, vWF • Plastic matching refractive index of glass. • Assay cell sample volume <10 uL per channel	• **Immunostaining** • **Confocal microscopy** • Single cell / platelet analysis • Cell rolling and adhesion studies • Thrombosis • Cell proliferation
Vena8 Endothelial+™	• Brightfield / phase contrast • 20X, 40X, 60X magnification	• **Easy to culture monolayers** with conventional incubator.	• **Cell-cell interaction** • **Endothelial / epithelial cell monolayer culture** • Cell rolling and adhesion studies.
VenaEC™	• Brightfield / phase contrast / fluorescence • 20X, 40X long working distance • Low fluorescent background	• **Easy to culture monolayers** with conventional incubator & 6-well plates. • Ability to use different substrate materials.	• **Cell-cell interaction** • **Endothelial / epithelial cell monolayer culture** • Cell rolling and adhesion studies

cellix

Cellix Ltd., Unit 3.81,
Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's Street, Dublin 8, Ireland.

Tel: +353 (0)1 896 4016
Fax: +353 (0)1 896 2771
Email: info@cellixltd.com
URL: www.cellixltd.com

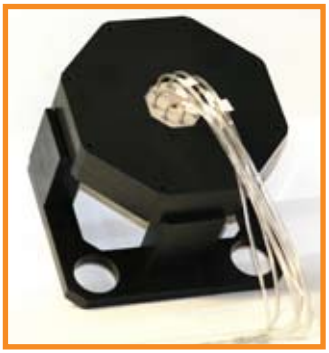
Cellix's Microfluidic Pumping Technology

Customised solutions to fit your needs

Mirus™ Nanopump

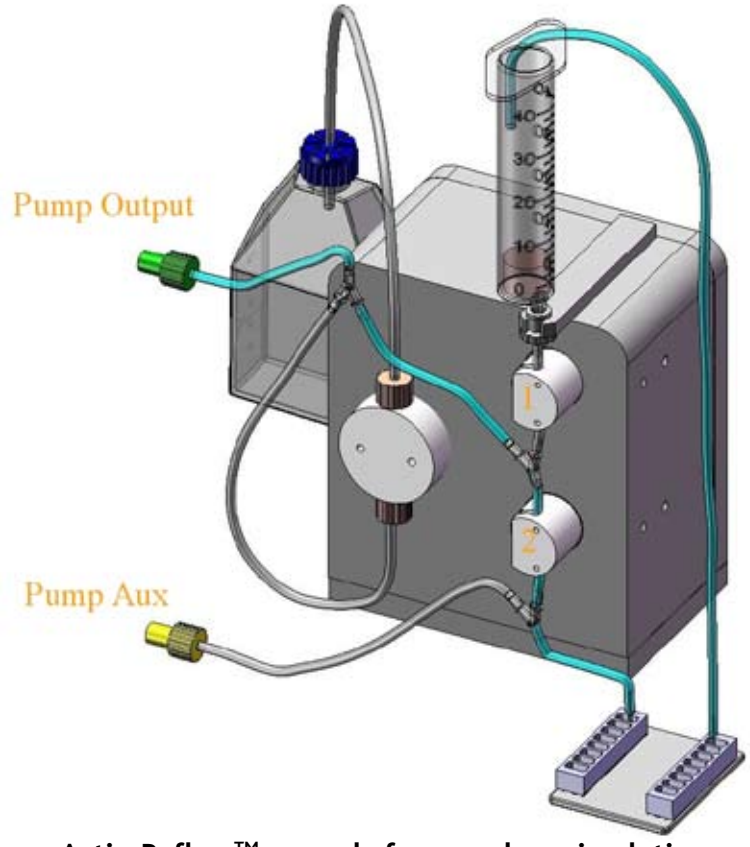
Mirus™ Nanopump is a patented, precision, microfluidic, **8-channel syringe pump** for cell analysis under shear flow mimicking physiological flow in the human vasculature. Includes **MultiFlow8™**, a manifold that enables the flow from the Mirus™ Nanopump to be split equally into 8 separate tubes to conduct 8 assays simultaneously in the Vena8™ / Vena8 Fluoro+™ / Vena8 Endothelial+™ Biochips, resulting in higher throughput with this **8-channel syringe pump**. Mirus™ Nanopump is PC-controlled by ***FlowAssay™*** Software.

Mirus™ Nanopump 2.0



MultiFlow8™

More accurate and reliable than pneumatic-driven flow!



Activ-Reflow™ upgrade for sample recirculation

Features

- Ideal for getting started with shear flow experiments
- Compatible with inverted microscopes.
- Upgradeable to VenaFlux™ Platform.
- Higher throughput than traditional syringe pumps: With MultiFlow8™, can run 8 assays in parallel.
- High (up to 450 dyne/cm^2) and low (down to 0.05 dyne/cm^2) shear flow rates achievable with 1 mL and 100 µL syringe sizes, respectively.
- Compatible with wide range of cell suspensions and whole blood.

Upgrades such as **Activ-Reflow™ for sample recirculation** broadens applications for users such as analysis of **thrombi formation** with small sample volumes or **biofilm culture** over extended periods of time (e.g. weeks).

Cellix can customise a solution around your existing microscope – ask for more details at ***sales@cellixltd.com***

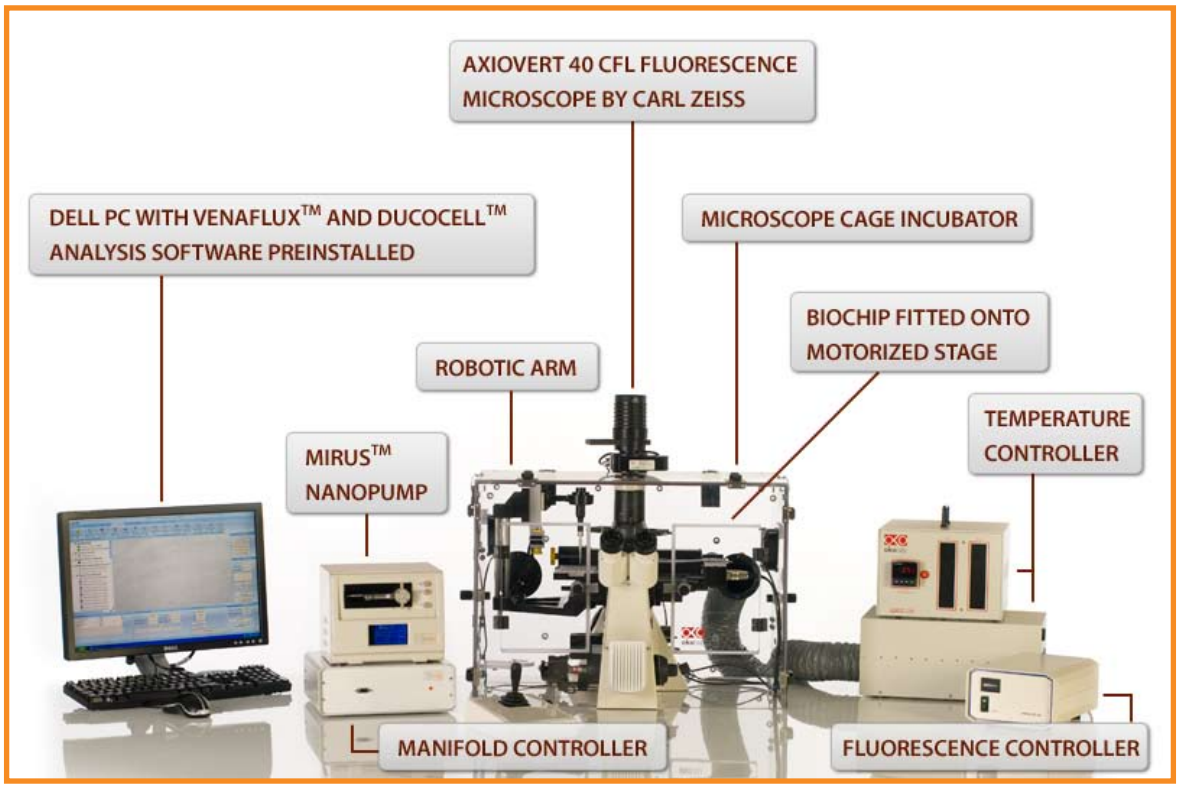
cellix

Cellix Ltd., Unit 3.81,
Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's Street, Dublin 8, Ireland.

Tel: +353 (0)1 896 4016
Fax: +353 (0)1 896 2771
Email: info@cellixltd.com
URL: www.cellixltd.com

VenaFlux™ Platform

For higher throughput requirements of cell-based assays under shear flow; the **VenaFlux™ Platform** will meet your needs. The VenaFlux™ Platform is the first semi-automated microfluidic platform capable of executing cell rolling, binding/adhesion and migration studies under shear flow mimicking *in-vivo* flow rates.



Ideal for:

- Functional cell-ligand and cell-cell interaction studies.
- Morphology analysis of cell adhesion and migration.
- Tracking of flowing, rolling and migrating cells.
- IC50 curves easily produced.
- Preclinical drug development and lead optimization.

Benefits:

- Robust and easy to use.
- Ideal for use with whole blood for platelet adhesion, aggregation and thrombi formation (thrombosis); bio-film culture over extended periods of time.
- Easy to study cell adhesion, migration and transmigration – more physiologically relevant than static assays!

VenaFlux™ Robotic Arm integrates with 96-well plates.

cellix

Cellix Ltd., Unit 3.81,
Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's Street, Dublin 8, Ireland.

Tel: +353 (0)1 896 4016
Fax: +353 (0)1 896 2771
Email: info@cellixltd.com
URL: www.cellixltd.com

The VenaFlux™ Platform includes:

Flow Apparatus compatible with Cellix Biochips:

- Mirus™ Nanopump 2.0 for sample shear flow control; controlled by PC VenaFluxAssay™ software
- Activ-Reflow™ for sample recirculation
- Biochip Frame holder

Automated Microscopy Workstation:

- Zeiss Axiovert Microscope with Fluorescence Module
- Temperature Control: Microscope Cage incubator with fluorescence black-out panels.
- Digital camera – Cellix recommends cameras from DeltaPix; QImaging or Hamamatsu depending on user requirements
- Motorized Stage and controllers for Microscope – from Marzhauser or Ludl
- VenaFlux™ Robotic arm for sample dispensing – **integrates with 96-well plate format**.
- VenaFluxAssay™ Software and PC – **integrates and automates image capture from digital camera; motorized stage movement; Mirus™ Nanopump and VenaFlux™ Robotic dispensing arm.**

Image Analysis Software:

- DucoCell™ software for image analysis: cell counting, morphology etc.
- Image Pro Analyzer for cell tracking analysis: rolling cells (i.e. speed) etc.

Cellix's Imaging Options: Data analysis made easy!

Choose the right camera...

Image quality is dependent on the quality of your camera. Depending on your application; Cellix can recommend the best camera for your needs. Cellix supplies high quality cameras from reputable suppliers such as **DeltaPix, QImaging and Hamamatsu.**
This is a guide for choosing the right camera but Cellix will assist you in choosing one that will meet your needs:

Camera Model	Resolution max, pixels	Frame Rate, fps	Applications
DeltaPix 450D	• 1616x1216 • 8 bit colour	12fps at 1616x1216 22fps at 800x600 30fps at 400x300	Brightfield, High level fluorescence, Color Imaging
QImaging Rolera XR	• 696x520 • 12 bit monochrome	20fps at 693x520 40fps at 346x270	Low level brightfield and fluorescence, High speed imaging
QImaging Retiga 2000R	• 1600x1200 • 12 bit monochrome	10fps at 1600x1200 20fps at 800x600 40fps at 400x300	Low level brightfield and fluorescence, High resolution and speed imaging
Hamamatsu ORCA 03	• 1344x1024 • 12 bit monochrome	8fps at 1344x1024 16 fps at 672x512 28 fps at 327x256	Low level brightfield and fluorescence
Hamamatsu ORCA R2	• 1344x1024 • 12 bit monochrome	16fps at 1344x1024 28 fps at 672x512 45.7 fps at 327x256	Low level brightfield and fluorescence

Cellix Ltd., Unit 3.81,
Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's Street, Dublin 8, Ireland.

Tel: +353 (0)1 896 4016
Fax: +353 (0)1 896 2771
Email: info@cellixltd.com
URL: www.cellixltd.com

Cellix's Imaging Options: Data analysis made easy!

Choose the right software...

DucoCell™ Image Analysis for:

- Fast and reliable cell counting and analysis of cell morphology.
- Automatic – wizard feature for fast analysis.
- Morphology analysis of cell area, diameter, form-factor (e.g. for cell spreading on adhesion) and more.
- Ability to analyse sub-populations using cell sorting/filtering feature.
- Data are easily exported to Excel spreadsheet with automatic graph generation (e.g. IC50 curve for % cell adhesion vs. shear flow rate).

DucoCell Analysis Software

Image-Pro Plus Analysis Software

Image-Pro Plus Software for:

- Cell tracking – analysis of movies (*.avi etc.) for cell flow, rolling and migrating cells.
- Analysis of time-lapse movies.

Supplied in partnership with:

MediaCybernetics®
From Images to Answers®

If you would like additional information on any of our products, please contact us at:

European Headquarters:

Cellix Ltd.,
Unit 3.81, Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's St., Dublin 8, Ireland.
Tel: +353 (0)1 896 2799
Fax: +353 (0)1 896 2771
Web: www.cellixltd.com
Email: info@cellixltd.com

US Sales Office:

Cellix Inc.,
410 Park Avenue,
15th Floor, #935pmb,
New York, NY 10022, USA.
Tel: +1 (212) 231 8274
Fax: +1 (917) 210 8182
Web: www.cellixltd.com
Email: info@cellixltd.com

European Headquarters:
Cellix Ltd.,

Unit 3.81, Institute of Molecular Medicine,
Trinity Centre for Health Sciences,
James's St., Dublin 8, Ireland.

Tel: +353 (0)1 896 2799
Fax: +353 (0)1 896 2771
Web: www.cellixltd.com
Email: info@cellixltd.com

USA Headquarters
Cellix Inc.,

Cellix Inc.,
410 Park Avenue, 15th Floor, #935pmb,
New York, NY 10022, USA

Contact: Julia Umlauf
Tel: + 1 (917) 623 4456
Web: www.cellixltd.com
Email: j.umlauf@cellixltd.com

cellix