



GALILEO TMA CK 4500

HTS Tissue Microarray Platform

Tissue Microarray (TMA)

"A Block Of Samples From Hundreds Of Blocks"

(S. M. Hewitt, M.D., Ph.D., Tissue Array Research Program, LP, CCR, NCI, NIH)

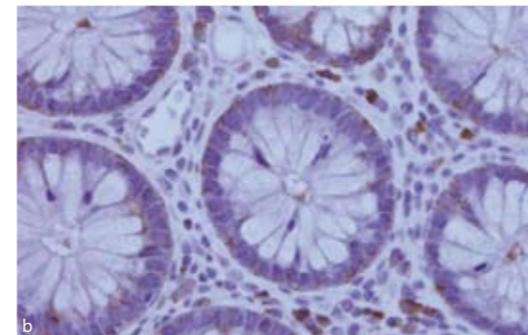
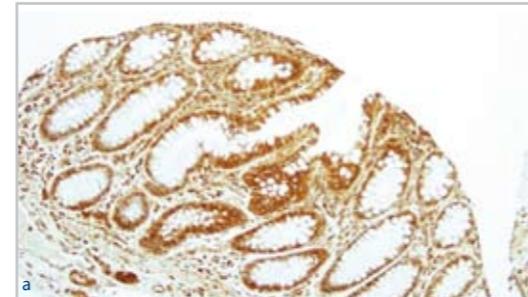
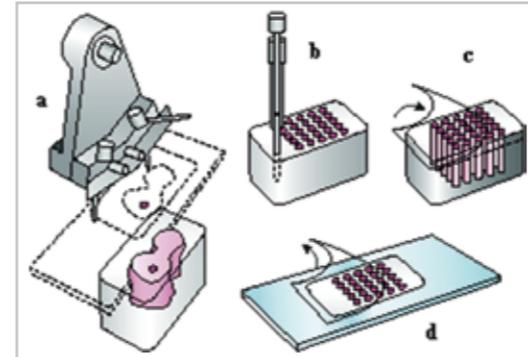
TMA technology

The TMA technology is a technical procedure that combines **tens to hundreds of paraffin-embedded tissue specimens into a single paraffin block**. Cylindrical tissue cores (typically 0.6-2.0 mm in diameter) are acquired from one or more representative regions of a paraffin-embedded tissue block (**donor block**) and then precisely arrayed into a new "**recipient**" paraffin block, using a custom-built instrument. Up to 200 consecutive sections of 4-5µm thickness can be cut from each TMA block, mounted on a microscope slide and **processed like ordinary tissue sections with a wide range of techniques** (histochemical staining, immunohistochemical and immunofluorescent staining, FISH).

TMA advantages

The use of relatively **small tissue cores**, positioned onto a unique slide, allows **simultaneous histological analysis of hundreds of samples**.

- High throughput screening of expression
- Reduction of the number of slides to be mounted and analyzed → Time saving
- Reduction of the amount of reagents and antibodies used → Money saving
- Analysis of various biological specimens under uniform reaction conditions → Increasing the consistency of the results
- Saving of precious biological samples and possibility to re-use donor blocks



(a) Normal colon with decreasing staining ascending the crypt, 10X
(b) Normal colon base of crypt (proliferative zone) with cytoplasmic staining, 40X

Applications

CELL MICROARRAY (CMA)

CMA evolved from TMA with the exception that **cells are pelleted**. Cells are grown in culture, fixed, suspended in agarose and embedded in paraffin.

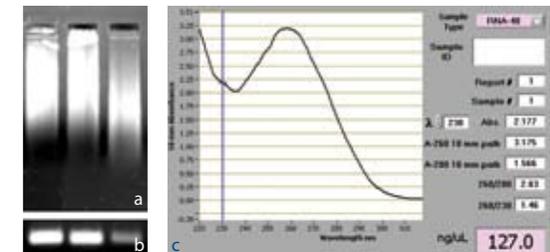
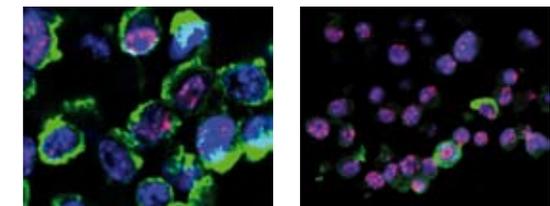
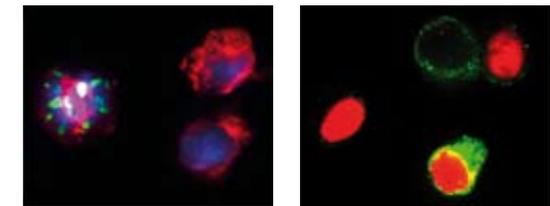
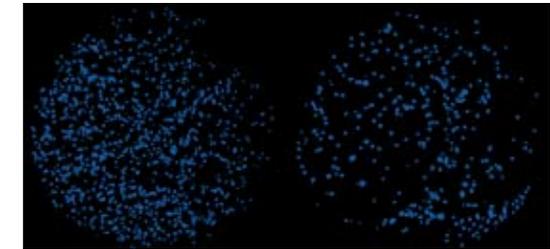
- Immunocytochemistry analysis (IC) of any type of cells
- High-throughput screening of hundreds of cell samples on a single slide
- Several slides can be generated from a paraffin recipient block ready to be assayed with different markers

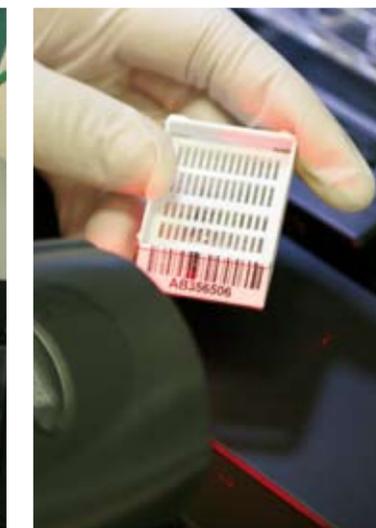
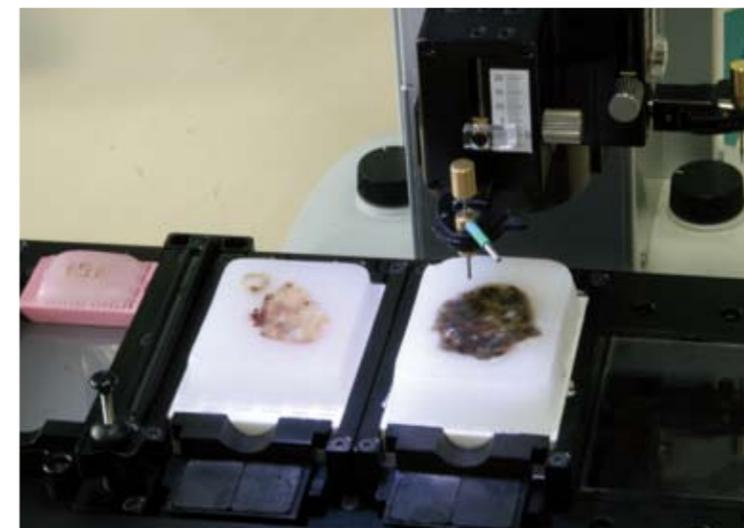
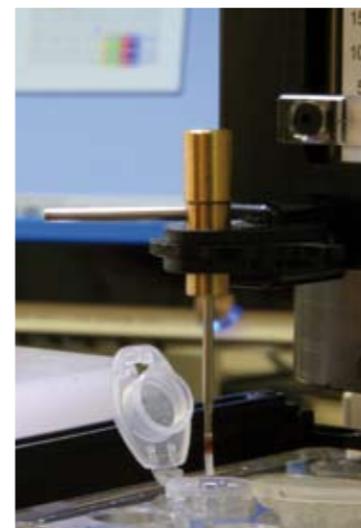
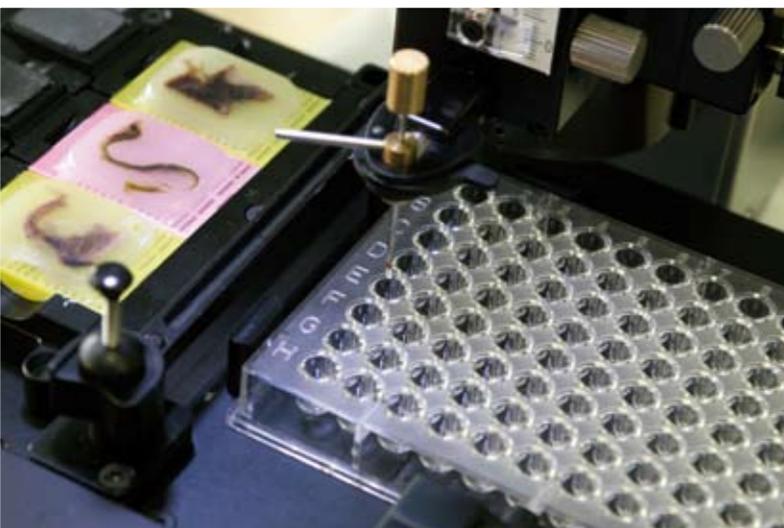
TISSUE CORE PICKING & DISPENSING

Galileo 4500 can be used for TMA, CMA and as **sample picker for nucleic acids (DNA and RNA) extraction and for protein isolation using appropriate fixative**.

The tissue cores extracted from precise regions of interest may be dispensed into dedicated vessels for further processing such as DNA, RNA, miRNA and protein extraction. Microfuge vials, strips or microtiter plates may be used, depending on the downstream applications. **Parallel analyses on the same specimen** can be performed simultaneously.

DNA was extracted from tissue cores (1 mm size and 3.5 mm³) from human prostate, uterus and colon using silica based matrix (Malferrari et al., 2002). The agarose gel (0.8%) shows the quality of DNA extracted (a) and the PCR amplification using 100 ng of DNA with primers specific for the BRCA1 gene (b). RNA was extracted from prostate tissue core and quantified by Nanodrop (c). The expression of the housekeeping genes GADPH, 18S RNA, RPL13a, MRPL19, ACTB was assessed by Taqman and the expression of miRNA has-miR-21 and has-let7a was verified.





Galileo TMA CK 4500: unique features

"PICK & DISPENSE" OPERATING MODE

- Tissue core dispensing into dedicated vessels (**microtiter plates, strips, microfuge vials**) for downstream application such as DNA, RNA and miRNA extraction.

HOLDER FOR MACRO-BLOCKS AND CUSTOM SIZE PARAFFIN BLOCKS

- Possibility to work with up-to-three standard macro-blocks. Macro-blocks may be used both as donor and recipient. For example, macro-recipient blocks may be used to compare simultaneously a high number of samples.
- Possibility to work with custom size paraffin donor blocks (up to 120 x 85 mm).

USER FRIENDLY DEDICATED SOFTWARE (ISETMA SOFTWARE)

- Equipment interface designed to assist the user in all operating phases of TMA project, from the building of the array pattern to the final reporting for specimen tracking.
- Generation of a report on an Excel spread-sheet and an XML report to interface with other equipments, like digital robotic scanners.
- Two dedicated functions that help the user in the identification of the punch areas on the donor blocks performing only one alignment for each block instead of one alignment for each spot, as in the classical procedure.
- Modified interface for "Pick & Dispense" operating mode.

1D & 2D BARCODE READER

- Import of the donor block codes that will be used in the TMA project.
- Check of the donor block codes during the sampling phase to avoid mismatching that could compromise TMA quality.
- Define, import and check the tubes used during "Pick & Dispense" operation.

HIGH SPEED AUTOMATED TRAY WITH MODULAR SAMPLE HOLDER

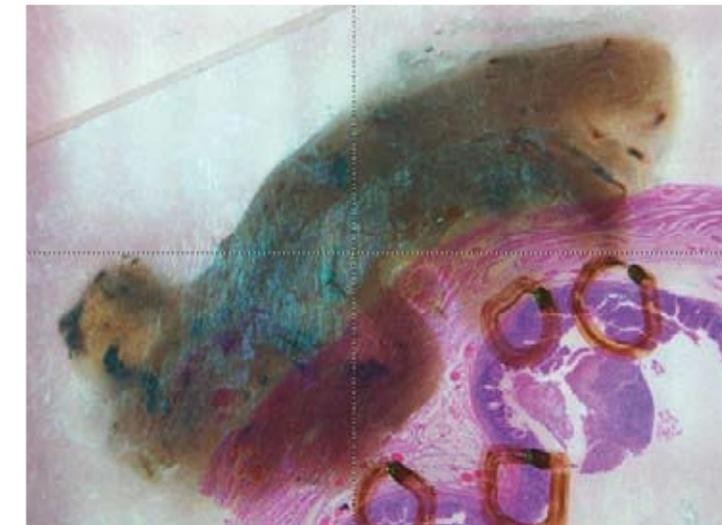
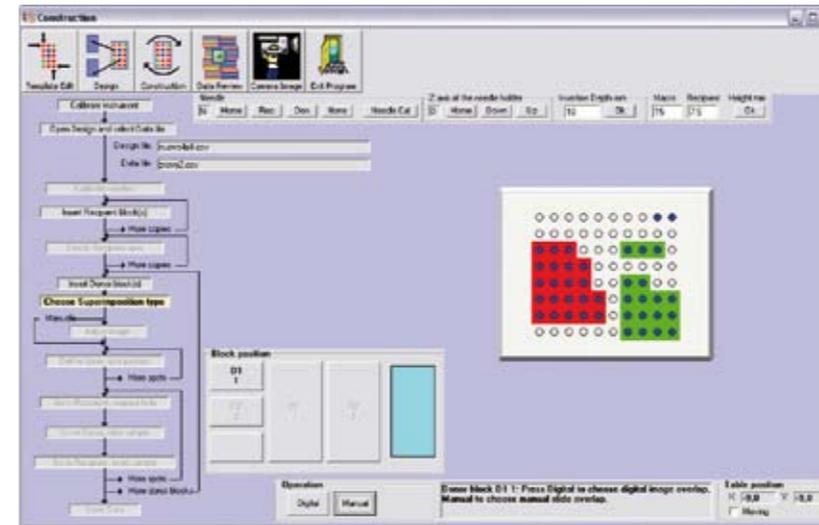
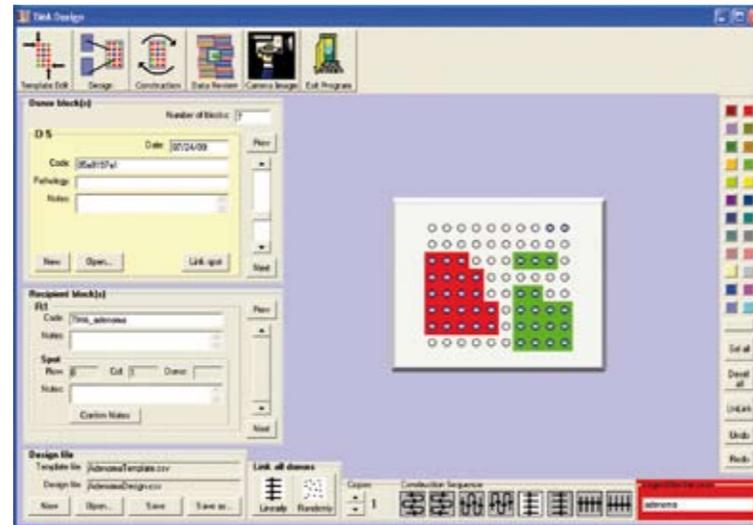
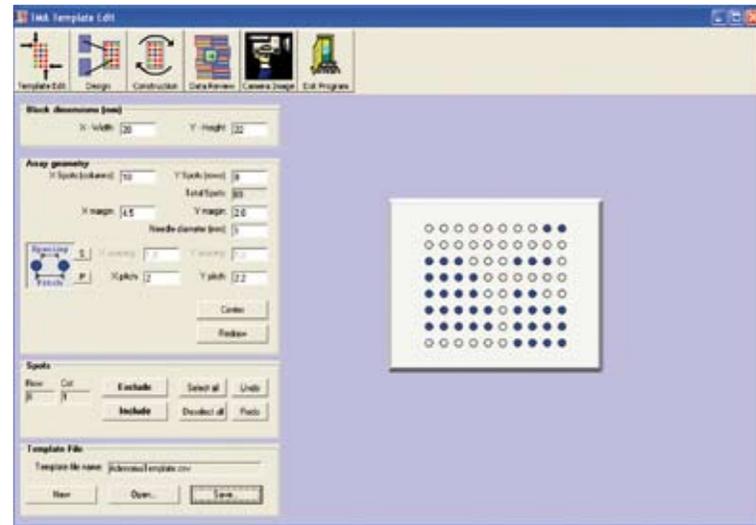
- Computer assisted and automated tray that ensures precise and rapid positioning of the paraffin blocks while in use.
- Modular sample holder that allows positioning of various specimen items: standard histological cassettes, macro-blocks, custom size paraffin blocks, microplates or microfuge vials.
- Dedicated holder designed according to microplate standard.
- Dedicated glass slide holders to capture digital images of both standard and macro glass slides.

HIGH RESOLUTION DIGITAL CAMERA

- Easy identification of the punch areas on the donor blocks. The operator looks at the images of the donor block and of the pre-marked reference slide on the PC monitor.
- Possibility to save the images of the slides and of the paraffin blocks on the computer HD for later use during virtual alignment in the punch area selection and for digital reports.

LED BACK-LIGHT AND REFLECTED ILLUMINATION

- Double source illumination of the paraffin blocks as well as the glass slides to improve the quality of the images.
- Independent regulation of the two light source intensity.



The IseTMA software

TEMPLATE EDIT

- Create the geometry of the array (paraffin size, needle diameter, number of spots and spacing or pitch between the spots).
- Automatic software check of data inconsistencies or errors.
- Introduce rows/columns or spot groups of spacing to separate the various specimens.
- Automatically centre the array in the recipient block.
- Check the template directly on the recipient block
- Define a library of TMA templates to be re-called and re-used in different TMA projects.

DESIGN

- Define the donor blocks to be used. Donor blocks can be inserted once or added during the job progression.
 - Import the donor block codes by using the barcode reader.
- Link each donor block to one or multiple spots of the TMA.
 - Manual spot link
 - Linear spot link
 - Random spot link
- Define replicates of the created TMA. Up to 8 replicates can be performed. The maximum number of replicates depends on the mounted configuration.
- Add a text note for each donor block, recipient block or spot. These notes will be copied in the final report.
- Associate a color code to each spot to improve TMA reading.

CONSTRUCTION

- Follow the flow chart to proceed in the TMA construction.
- Check the template directly on the recipient block.
- Save all the punch positions for each donor block at the same time. The arrayer will automatically place the blocks during the sampling.
- Check the donor blocks during the sampling phase to avoid mismatching.
- Advance from one step to the next with a simple click of the mouse. All the block movements are automatic.
- Adjust errors and position mismatches in any moment with dedicated functions.
- Complete your array once in a time or later in different job sections.

PUNCH AREA SELECTION: MANUAL OVERLAPPING

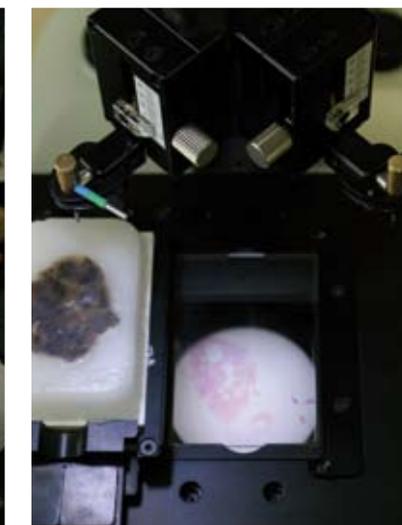
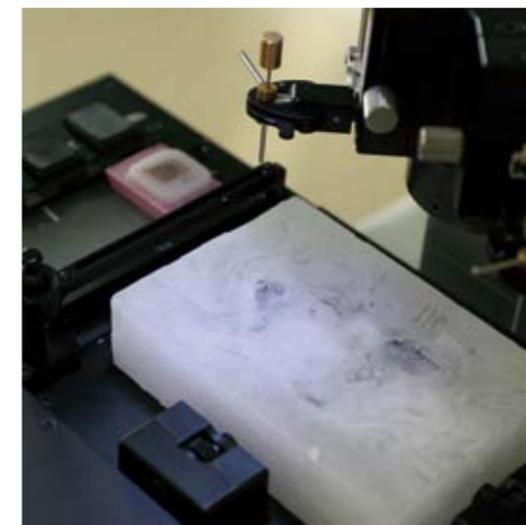
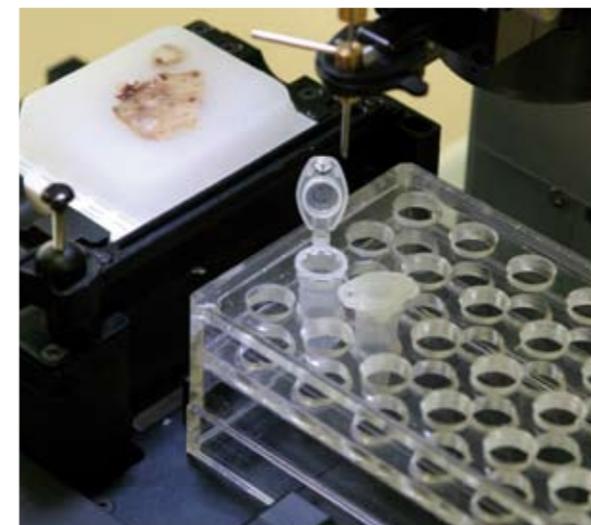
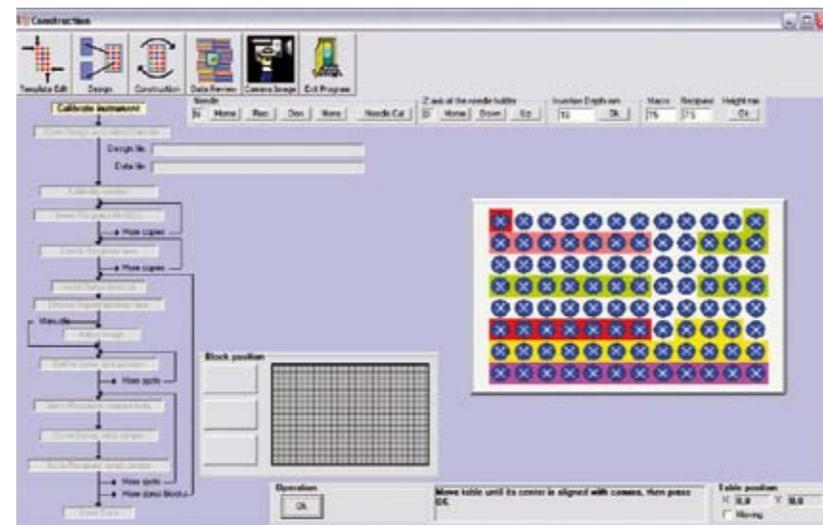
- Align the pre-marked glass slide with the donor block by looking the zoomed images on the LCD color monitor.
- Once aligned, save the punch positions using a joystick or with a mouse click.

PUNCH AREA SELECTION: DIGITAL OVERLAPPING

- Overlap the digitized pre-marked glass slide to the live block image.
- Modify the transparency of the digitized slide to better see the donor block morphology.
- Align the images rotating the camera and using specific commands (digitized image transparency, drag & drop of the digital image, etc.).
- Once aligned, save the punch positions with a simple mouse click. Target icon marks will be drawn in scale and IseTMA software will automatically check that no overlapping occurred.

ROW	1	2	3	4	5	6	7	8	9	10
1									Donor 1 CTRL	Donor 1 CTRL
2										
3	Donor 2	Donor 2	Donor 2			Donor 2	Donor 2	Donor 2		
4	Donor 2	Donor 2	Donor 2	Donor 2						
5	Donor 2	Donor 2	Donor 2	Donor 2						
6	Donor 2									
7	Donor 2	Donor 2								
8	Donor 2	Donor 2								
9						Donor 2	Donor 2	Donor 2	Donor 2	Donor 2
10						Donor 2	Donor 2	Donor 2	Donor 2	Donor 2

Color Legend
■ Donor
■ Normal tissue



DATA REVIEW

- Create the final report of the array on an Excel spread-sheet.
 - Each spot is properly identified during subsequent analysis.
 - Relate each spot to the corresponding information.
 - Personalize the report, for example adding the results of the analysis or images of the donor blocks and the spots.
- Create the final TMA report as standard XML TMA description file.
 - Interface with other equipment (like digital robotic scanners) is possible.

"PICK & DISPENSE"

- Define the recipient vessel used (microplate format, microfuge vials).
 - Import the recipient codes by using the barcode reader.
- Link each donor block to one or more position of the recipient rack.
 - Manual link
 - Linear link
- Define the number of cores per each recipient position.
- Check the donor blocks and the recipient vessels during the sampling phase to avoid mismatching.
- Save all the punch positions for each donor block at the same time, as in TMA construction.
- Advance from one step to the next with a simple mouse click. All stage movements and needle selection are automatic.

Galileo TMA CK 4500 flexibility

The modular sample holder mounted on the automated tray allows various configurations of the following items. Changing among various configurations can be easily performed by the user. Unscrewing and screwing modules is a matter of minutes.

STANDARD HISTOLOGICAL CASSETTES

- Possible mounting of up to 9 standard histological cassettes.

MACRO-CASSETTES

- Possible mounting of up to 3 standard macro-cassettes.

MICROTITER PLATES

- Dedicated holder designed according to microplate standard.

MICROFUGE VIALS

- Dedicated holder for up to 24 microfuge vials (1.5 or 2.0 ml).

CUSTOM SIZE PARAFFIN BLOCKS

- Possible mounting of one custom size paraffin block (up to 120 x 85 mm).

STANDARD AND MACRO GLASS SLIDES

- Holder for both standard and macro glass slide to capture digital images.

Technical specifications

HIGH RESOLUTION OPTICS

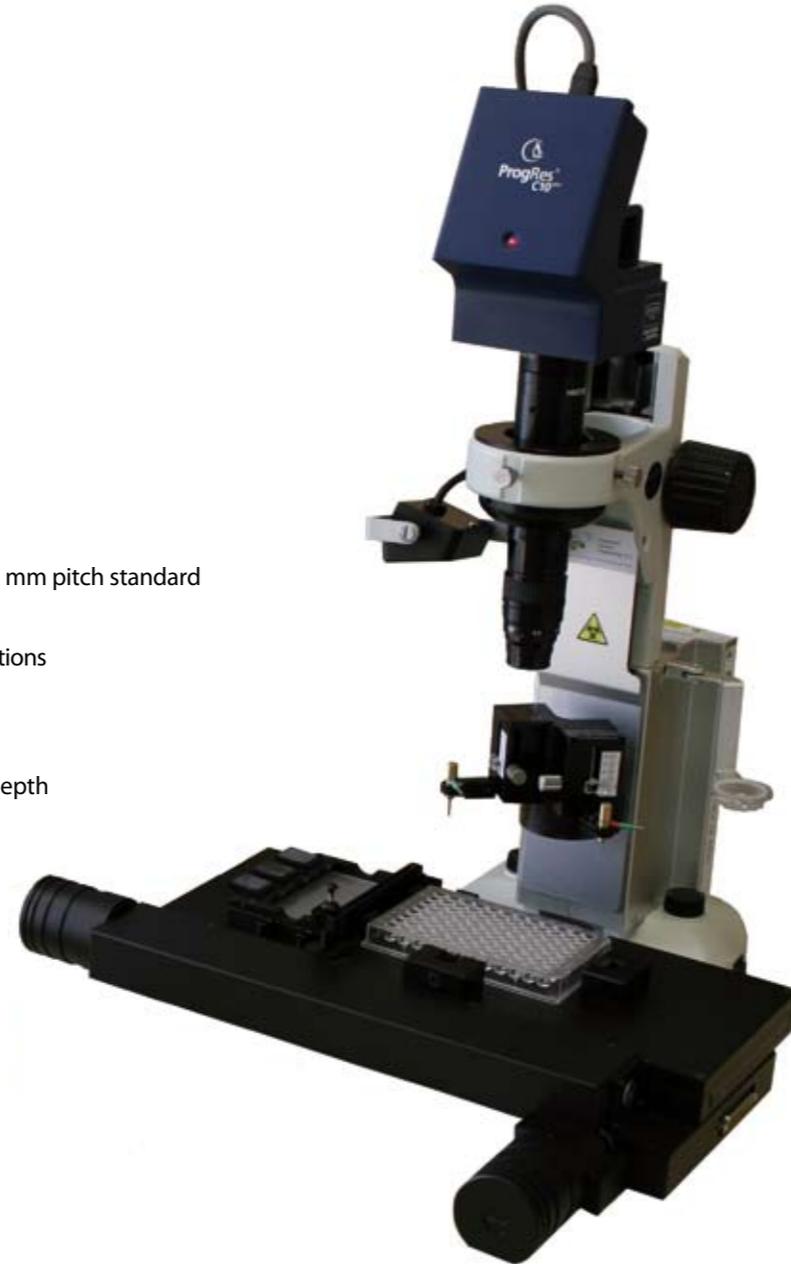
- Optics: Navitar Zoom6000
- Jenoptik ProgRes™ C3 camera
 - CCD sensor: 1/2" 3.3 Megapixel Color CCD
 - Pixel size: $3.45 \times 3.45 \mu\text{m}^2$
 - A/D Conversion: 3×12 bit RGB
 - Image resolution: up to 2080×1542 pixels
- 42X magnification optic with a 20" wide display
- Adjustable LED back-light and reflected illumination

AUTOMATED STAGE

- Prior Scientific H138 stage
 - Repeatability: $\pm 1 \mu\text{m}$
 - Stage speed: up to 60 mm/second
 - Drive screws: Zero backlash, recirculating ball screws; 2 mm pitch standard
 - Travel range: 240×77 mm
- Modular sample holder to allow various specimen configurations

NEEDLE HOLDER

- Automated and computer guided needle positioning
- Accurate and automated regulation of the core insertion depth
- Easy needle mounting

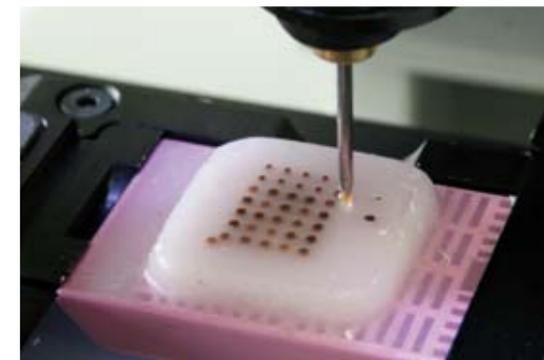


1D & 2D BARCODE READER

- Handheld barcode reader to read barcodes on histological cassettes and vessels
 - CCD digital scanning technology
 - Combined functionality: supports for all major 1D, PDF and 2D symbologies
 - Omni-directional scanning and wide working range to eliminate the need to align item and scanner
 - Laser-generated aiming pattern to enable quick and accurate data capture
 - Appropriate stand to switch between hands-free and handheld modes

NEEDLES

- Set of two punches: one for the donor block and another one for the recipient block
 - Standard (STD) needles
 - Macro needles: longer needles to sample into paraffin macro-blocks with thickness up to 15 mm
- Available diameters:
 - 0.6 mm (*only STD needles*)
 - 1.0 mm; 1.5 mm; 2.0 mm
 - 3.0 mm; 5.0 mm (*on request*)





*Integrated
Systems
Engineering S.r.l.*

biotechnology & bioengineering



facilitating research worldwide

BIOREP is part of



INTEGRATED SYSTEMS ENGINEERING S.r.l.

Via Fantoli 16/15 - 20138 Milano Italy
Tel. +39 02 58029724 - Fax +39 02 58018471
E-mail: info@isenet.it
Web page: www.isenet.it

BIOREP S.r.l.

Via Fantoli 16/15 - 20138 Milano Italy
Tel. +39 02 58014369 - Fax +39 02 58018471
E-mail: info@biorep.it - customerservice@biorep.it
Web page: www.biorep.it