

InnoQuant

Fluorescence whole-slide scanner
for cell and tissue imaging



A unique digital slide technology using laser scanning and pixel by pixel detection

Innopsys has developed a unique technology to image whole-slide samples using a laser point excitation technique combined with a PMT detection.

Excitation process and scanning strategy

The 4 laser beams (375, 488, 561 and 640 nm) are spatially separated and focused on the slide surface. They simultaneously illuminate the sample point by point (Fig.1).

The scanning process along the Y-axis is generated by moving the specimen relative to the laser beam.

The four excitation spots continuously scan the entire width of the sample slide through a mechanical drive along the X axis.

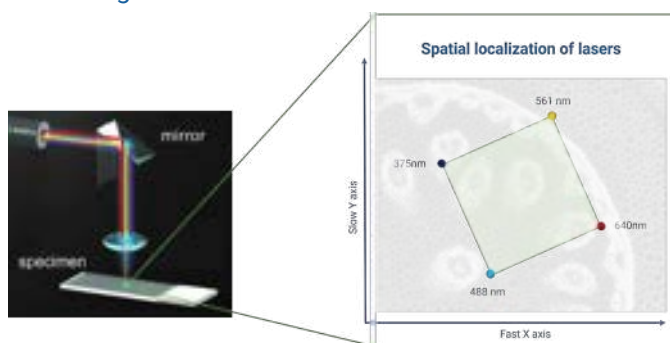


Fig 1. Spatial incidence of excitation lasers on the slide. The four lasers scan the sample simultaneously.

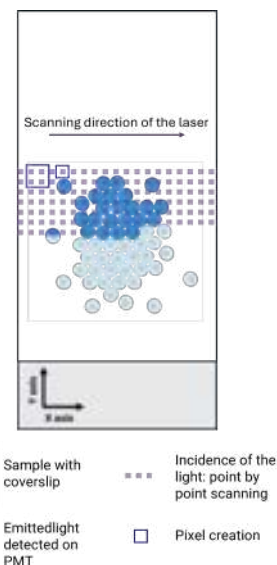


Fig 2. InnoQuant technology, scan in progress. The laser spot excites the sample point by point on the full width of the sample before scanning the next line. Each pixel is constructed depending on the size chosen by the user.

The scanning process along the Y-axis is generated by a movement of the sample holder relatively to the laser beam. The sample holder moves by increments with a step size depending on the chosen pixel size. Thus, each increment allows the lasers to scan the following line. This leads to a line-by-line continuous scan of the full length of the slide. There is no limit to the size or number of regions to be scanned on the slide (Fig. 2).

Detection principle and adjustable resolution concept

Considering that four spots are irradiated simultaneously by each excitation beam, the resulting fluorescence emission beams are detected instantaneously during the excitation scanning process. The emission beam is therefore directed towards a pyramidal-shaped mirror. Each fluorescence beam is reflected by one individual face of the mirror. Hence, each beam is guided towards its own detector. This described demultiplexing module guarantees that each detection path respectively corresponds to one excitation wavelength (Fig. 3).

The presence of a pinhole aperture on each emission channel ensures a depth filtering of the emission light coming from one irradiated plane of the sample.

Therefore, InnoQuant is an inherently depth-discriminating optical instrument, which gives it a significant advantage for 2D imaging of thick specimens.

Additionally, the 4 individual channels are equipped with an emission filter wheel located in front of the detector unit to increase spectral specificity and multiplexing options (Fig. 3).

Pixel-by-pixel digitization of the temporal intensity fluctuation is finally performed using 4 photomultiplier tubes (PMT) that convert photons into electron flux (Fig. 3). The resulting signal is periodically sampled by an analog-to-digital converter and thus transformed into a discrete, equidistant succession of measured data (pixels). Resolution number of pixels per unit area can be adjusted from 40 to 0.5 $\mu\text{m}/\text{pixel}$ according to sample requirements.

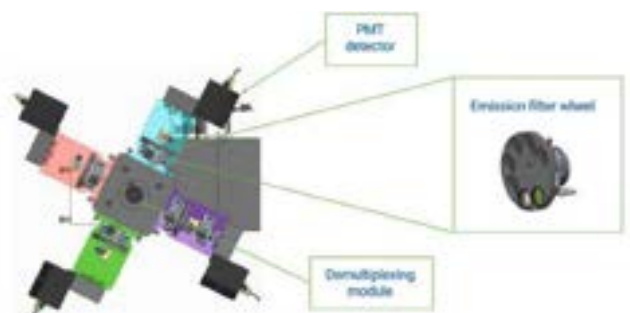


Fig 3. Optical design of the detection ensemble.

The final entire image is saved and available instantaneously on the disk and does not require any further fusion or shading correction operations (Fig. 6.B).

A unique digital slide technology to get rid of tiling and stitching issues

For convenience and availability reasons, regular automated imaging systems like widefield fluorescent microscopes or even LSM can also be used to image large specimen labeled with multiple fluorescent probes.

InnoQuant systems overcomes LSM underlying tiling bias (Fig. 4)

Laser Scanning Microscopy (LSM) uses line-by-line scanning of the specimen with a focused laser beam deflected in the X and Y directions by means of two galvanometric scanner lasers. Due to the use of a fixed magnification objective, sequential acquisition of several adjacent fields is required, with overlapping condition between two neighbor tiles to ensure a correct subsequent stitching operation.

Thus, some parts of the sample are illuminated twice, and detection of the fluorescence emitted by the specimen is also duplicated by the PMT. Inherently, illumination is not uniform across the sample, resulting in a bias in the signal collected relatively between each point. This raises a problem when it comes to conducting quantitative analysis based on fluorescence pixel intensities.

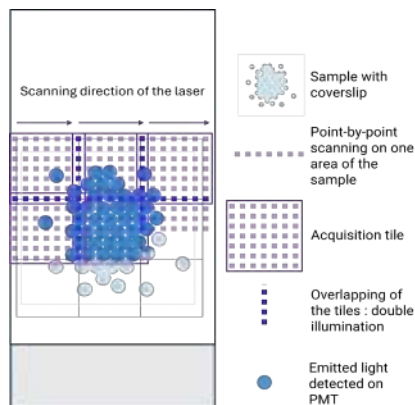


Fig 4. confocal technology, scan in progress. The laser spot excites the sample point by point along a line, until the image is complete. The final whole sample full image consists in several overlapped acquisition tiles.

InnoQuant overcomes widefield out-of-focus light capture, tiling bias and photobleaching effect (Fig. 5)

In widefield technology, the entire thickness of the specimen is exposed to the light source. Emitted light derived from all the fluorescent structures in this volume is captured by a camera, including the in-focus and the out-of focus light.

Moreover, as the optics consist in a fixed magnification lens with a finite field-of-view, sequential acquisition of several adjacent fields is required to image large samples entirely. The light incidence being round-shaped and the 2D array sensor chip being a rectangle, some parts of the sample are necessarily illuminated twice. Thereby, the same uniformity problems described for LSM arise.

Finally, widefield systems suffer more from the photobleaching impact when creating the focus map. Not all tiles are subjected to additional light exposure to adjust the focus. Final merging of individual tiles often requires shading correction to overcome undesirable intensity variations (Fig. 6.A), skewing any subsequent pixel intensity analysis.

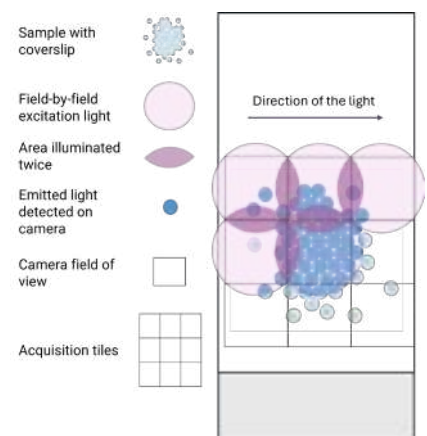


Fig 5. Widefield technology, acquisition in progress. The light illuminates the sample field-by-field and the emitted light is detected by a camera. The camera chipset allows the capture of rectangular acquisition tiles.

LSM/widefield vs InnoQuant (Fig. 6)

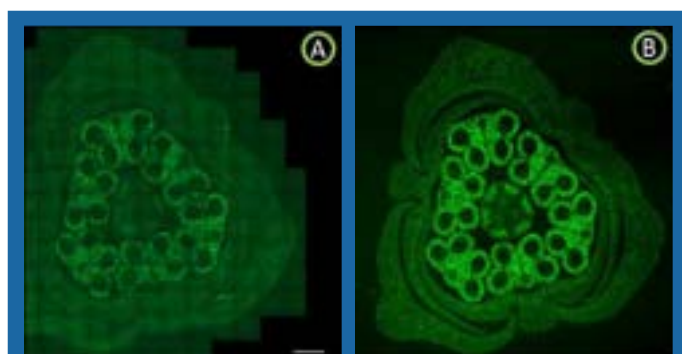


Fig 6. Autofluorescent lilyflower plant section (A) acquired with a camera-based slide scanner at a 20x magnification. Whole region scanned using 147 acquisition tiles. Stitching performed to generate the entire final image. **(B)** acquired with InnoQuant at 0.5µm/pixel. Whole region scanned at once. No tiling performed; final image has been generated on completion of scan.

Did you know?

How do other slide scanners on the market work?

Current commercial slide scanners are based on a 2D field-of-view detection, such as CCD or CMOS sensor technologies. Working with a synchronous field movement between the slide and the scanning stage, they rely on a tiling strategy to acquire whole specimen area.

What about scanners with confocal modality ?

Digital slide scanner solutions offering optical sectioning capabilities rely either on structured illumination or confocal spinning disk techniques. Both are camera-based imaging techniques and come with the same tiling issues described above..

- + Illumination homogeneity on the whole sample
- + No post processing (no shading correction)
- + Good signal/noise ratio
- + Fast scanning

Optics in InnoQuant

Optical pathway

The InnoQuant optical pathway consists in several components (Fig. 7).

It is made up of 4 fiber lasers. Each laser passes through a collimator before crossing a set of mirrors and reaching a dichroic mirror, or being reflected. Then, the laser beams pass through the focusing lenses before hitting the slide surface. The resulting fluorescence emitted light is directed through the dichroic mirror towards a pyramidal-shaped mirror, that reflects each emitted beam separately in 4 different directions. Then, the emitted light crosses successively a lens, an emission filter and a pinhole before reaching the photomultiplier tube where the signal is detected.

On the optical path of the 640 nm laser, one can find an additional polarizing beam splitter cube associated to a quarter waveplate. It permits to use the reflection of the laser beam on the slide for autofocus settings. This reflection is imaged by a CMOS sensor.

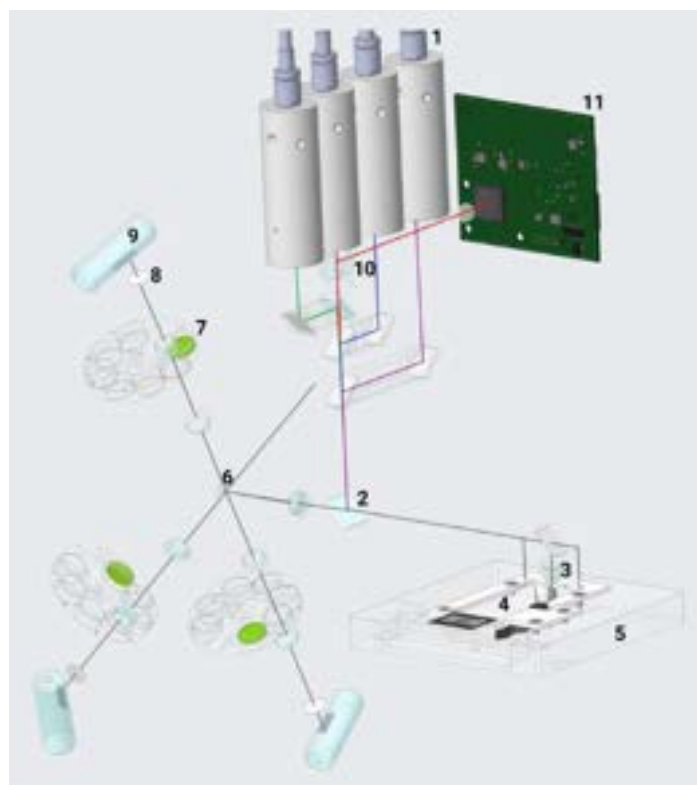


Fig 7. InnoQuant optical pathway, edited with Creo software.

1. Lasers, 2. Dichroic mirror, 3. Focusing lenses, 4. Specimen slide, 5. Stage, 6. Pyramidal mirror, 7. Emission filter wheel, 8. Pinhole, 9. Photomultiplier tube, 10. Polarizing beamsplitter cube + waveplate, 11. CMOS Sensor

Excitation and emission

InnoQuant has a dichroic mirror on its optical path, in order to separate excitation and emission beams. All emitted wavelengths comprised within the four transmission bandwidths can be detected on the corresponding detector (Fig. 8).

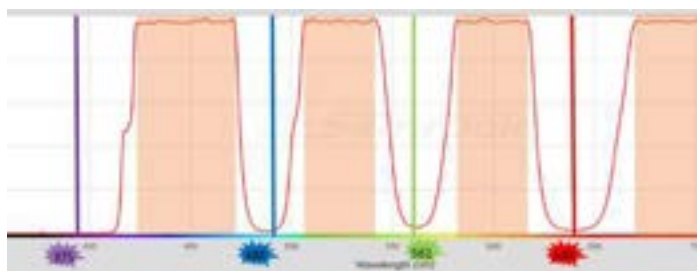
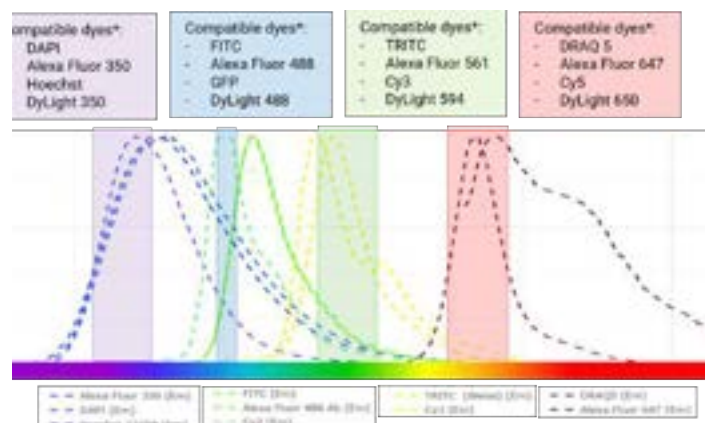


Fig 8. Dichroic mirror optical features. Dichroic mirror transmission bandwidth spectrum (red) with excitation wavelengths (vertical continuous lines). Orange regions represent the over 90% transmission bandwidths.



Standard emission filters

- FF01-440/40
- FF01-513/17
- FF01-593/40
- FF01-680/42

Fig 9. Standard emission filters.

Spectral match between common fluorophore emission spectra and InnoQuant standard filters, made with SpectraViewer. *Non-exhaustive list of compatible dyes

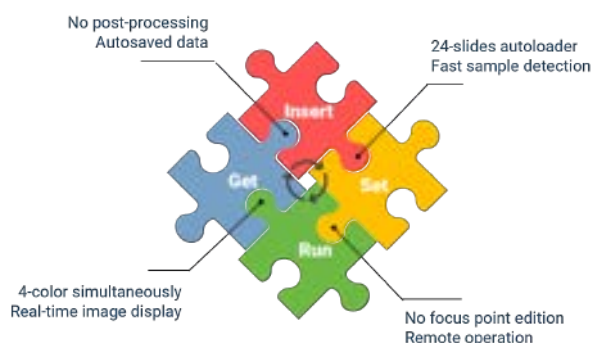
Quick time from slide insertion to results

Intuitive workflow



Time-saving automation at each step

The entire workflow of InnoQuant helps users of all levels to achieve results in just a few clicks. The scheme below shows all the functions in InnoQuant that save time compared to the competitors.



Customizable slide holder

Our single-slide version of InnoQuant can be adapted to accommodate specific sample holder dimensions. InnoQuant is not limited to standard microscopy glass slides. Our R&D team is adept at handling specific requests such as scanning mounted specimens thicker than 1.2 mm and/or larger than 26 x 76 mm (e.g.: microfluidic chips, imaging chamber, unconventional sample carrier, etc..)

Reproducible results



Monitoring of digital slide scanner performance is crucial in biomedical research.

This is why InnoQuant's delivery package includes a validation slide from Argolight®, enabling final user to assess his device quality throughout its life cycle.

A **Check Scanner procedure** can be launched via InnoQuant software to get quality control (QC) metrics such as intensity response, spectral crosstalk between channels, detection linearity, shift accuracy between channels or scanner/software communication. The QC test success relies on acceptance criteria for these values compared to Ex-factory reference values.

"Our Core is helping many researchers with whole slide immunofluorescent images within and outside of University of Colorado. Main challenge is time, needed for big tissue scans and photobleaching. InnoQuant solves both problems very elegantly. Additionally, interface is user friendly and doesn't take a lot of time to start."



Assistant Professor, PhD.
University of Colorado, Anschutz Medical Campus

Application fields

Cell morphology and characterization of organelles organization

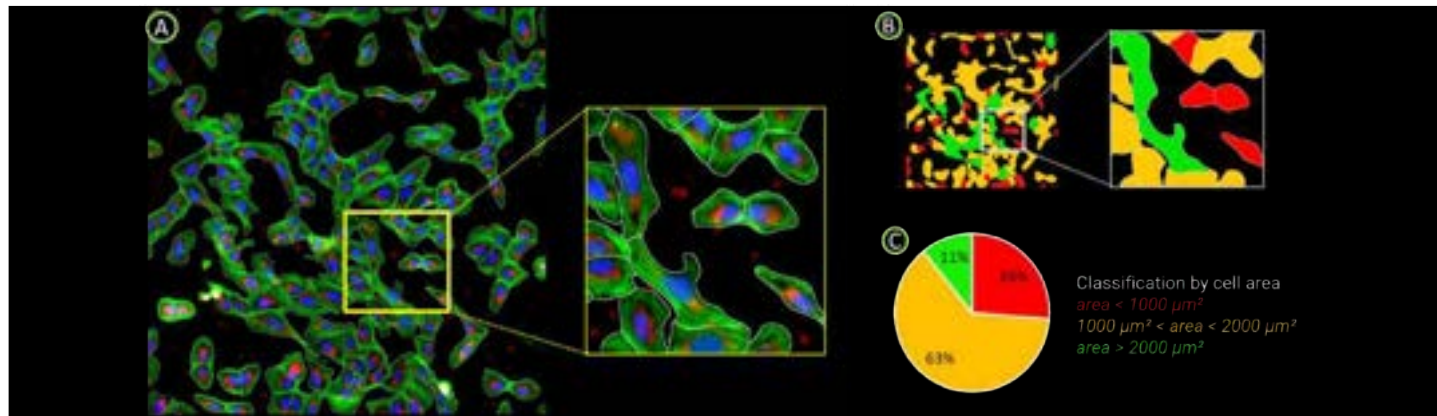


Fig 10. Immunochemistry stained endothelial cells fluorescence image and analysis. (A) Scan and ROI of the sample. (B) Cell segmentation based on cytoskeleton pattern. (C) Cell area distribution. Analysis conducted with ImageJ using CellPose DeepLearning segmentation. Labeled cell organelles: nuclei (blue), actine (green), stress fibers (red).

Cell morphology investigations are helpful to understand many cell growth mechanisms or to assess the impact of treatments on cell condition.

In this particular study, endothelial cells have been classified according to their area (Fig. 10). Individual cells have been divided into disjoint regions using a deep learning tool (CellPose) in ImageJ (Fiji) analysis software. CellPose requires two-channel input images to run a cellular segmentation based on some features of the nuclei and of the actine cytoskeleton such as pixel intensity, geometry and texture.

The cell-cultured sample was scanned with InnoQuant at a 0.5 $\mu\text{m}/\text{pixel}$ resolution. The results showed an uneven distribution of cells area, with the majority of cells between 1000 μm^2 and 2000 μm^2 .

Histological morphometry studies at the whole organ scale

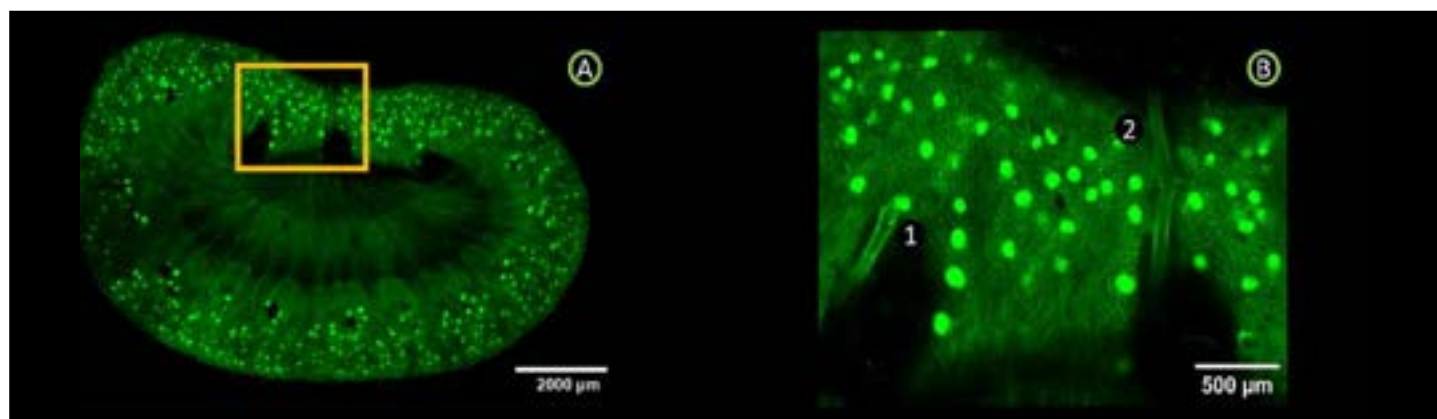


Fig 11. Cleared mouse kidney sample (550 μm thickness) Sunjin Lab Co FluoTissue®. (A) Scan and ROI of the sample (B) ROI and numbered blood vessels. Analysis conducted with ImageJ. Immunohistology fluorescent staining: Blood vessels– AF488

Examining images of whole organs can provide a great deal of information about their shape, size, development process and health. The kidney is vulnerable to a number of diseases such as acute kidney injury, which can lead to chronic kidney disease, and small vessel disease, characterized by microvascular dysfunction.

In this example, the specimen was scanned with InnoQuant at a resolution of 0.5 $\mu\text{m}/\text{pixel}$ (Fig. 11). The blood vessels are stained with AF488 and are particularly visible on Fig. 11. B. An analysis was performed with ImageJ to measure the mean diameter of the blood vessels identified on the ROI. The first blood vessel has a mean diameter of 85.57 μm and the second blood vessel has a mean diameter of 82.58 μm . These few data support a healthy vascular network hypothesis.

Application fields

Biomarker quantification in tissue microarrays (TMA)

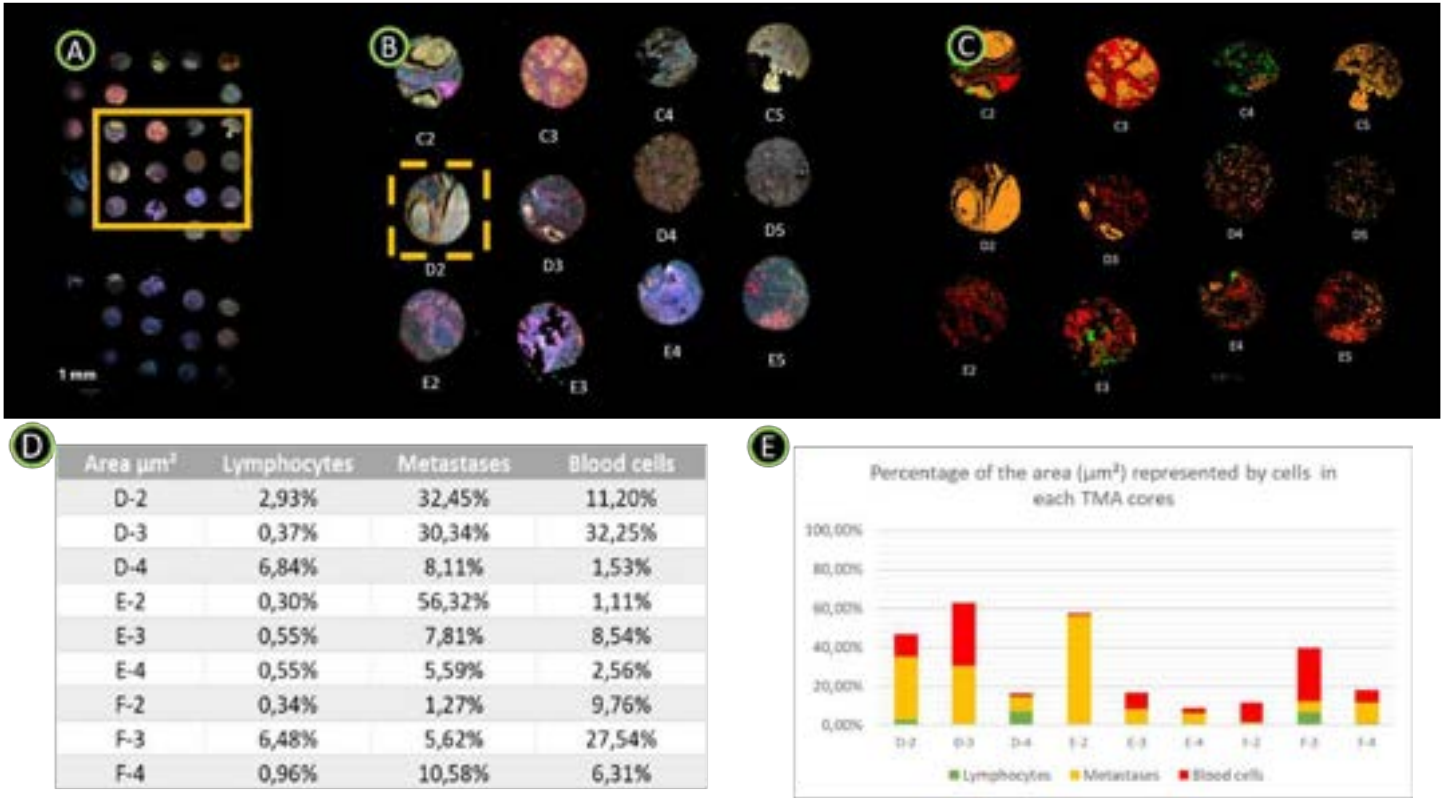
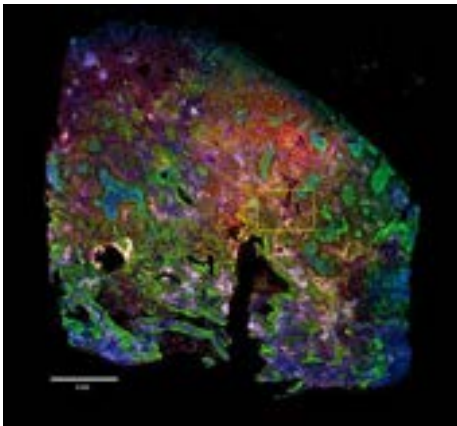


Fig 12. Breast cancer tissue microarray (TMA) image and analysis. (A) Scan and ROI of the TMA. (B) ROI of the TMA cores. (C) Detections of the cells of interest. (D) Classification of the cell type areas. (E) Percentage of the area represented by cells in each TMA cores. Analysis conducted with QuPath. Dyes: CD45 lymphocytes – AF488 / Lymph and metastases – AF555 / Vessels and fibroblasts – AF647

Tissue microarray (TMA) experiments deliver rapid immunohistochemical analysis of a great number of tissue samples in parallel. The goal of this study was to use breast cancer TMA to investigate spatial organization and distribution of cell types in breast cancer cores (Fig. 12). The immunostaining pattern highlighted the presence of lymphocytes, metastases, vessels and fibroblasts on each sample. TMA were scanned with InnoQuant at a 0.5 $\mu\text{m}/\text{pixel}$ resolution. The analysis was conducted with QuPath. The results showed a patient-dependant uneven distribution of cell types within the samples. Further analysis is required to better understand the causes of such differences.

Tumor microenvironment (TME) analysis



Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers. Use of therapeutic agents targeted to specific molecular features of the tumor has become standard practice. Therefore, investigating tumor tissue samples (Fig. 13) to characterize the presence of immune cells and immune checkpoints is helpful to target the appropriate therapy for individual patient treatment.

The goal of the following analysis, performed on QuPath, was to characterize the distribution of immune cells around the tumor areas.

Fig 13. Full image and ROI of NSCLC tissue section stained by multiplex immunofluorescence. Analysis performed on QuPath. Dyes: nuclei – DAPI / PDL1 – FITC / CD4 – AF594 / Granzyme B (NL cells) – Cy5

Application fields

Tumor microenvironment analysis in non-small cell lung cancer: characterisation of Natural Killer (NK) cells clusters

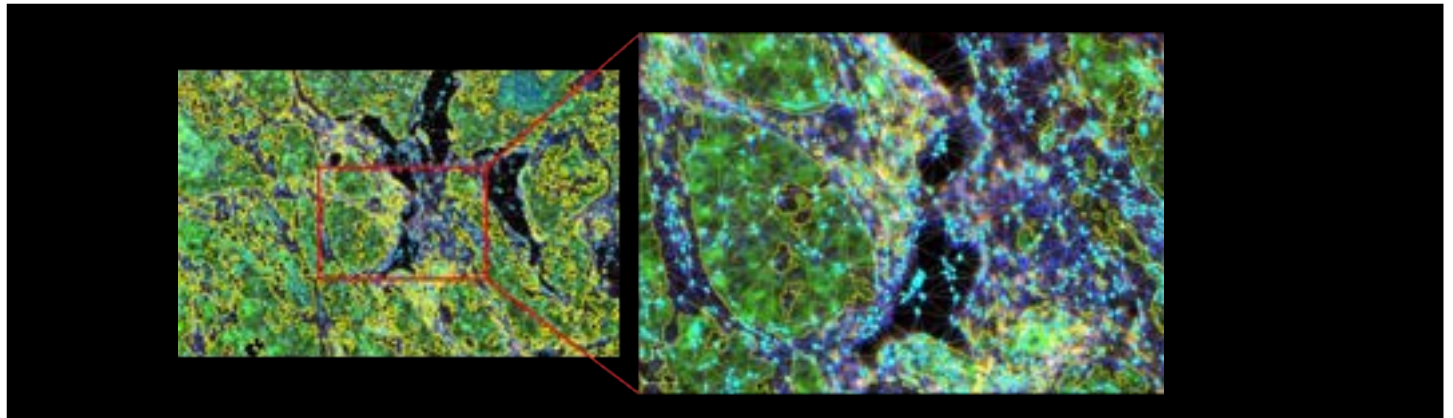


Fig 14. ROI and details of the NK cells clusters located in the microenvironment of the tumor cells area. Tumor cells area is outlined in yellow, NK cells are displayed in cyan. Analysis performed on QuPath using the Delauney clusters features 2D, threshold 100 μm
Dyes: nuclei – DAPI / PDL1 – FITC / CD4 – AF594 / Granzyme B (NK cells) – Cy5

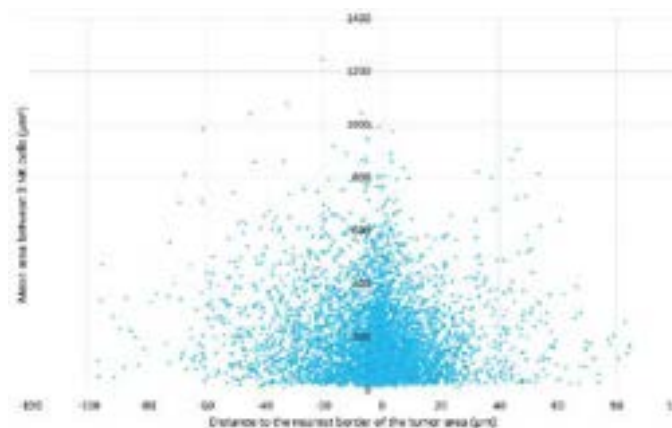


Fig 15. NK cells density plotted against their proximity to the tumor cells area

For a more detailed analysis, please refer to the application note *Tumor microenvironment analysis in NSCLC (non-small cell lung cancer) using InnoQuant scanner*, available at www.innopsys.com

References for applications

Ultrasound super-resolution imaging provides a noninvasive assessment of renal microvasculature changes during mouse acute kidney injury - PubMed (nih.gov)
Small vessel disease: Connections between the kidney and the heart - PubMed (nih.gov)
Insulin-like Growth Factor-binding Protein-3 in Breast Cancer: Analysis with Tissue Microarray | Anticancer Research (iijournals.org)
Bankhead, P. et al. QuPath: Open source software for digital pathology image analysis. Scientific Reports (2017). <https://doi.org/10.1038/s41598-017-17204-5>
Stringer C., Wang T., Michaelos M. & Pachitariu M. (2021). Cellpose: a generalist algorithm for cellular segmentation. Nature methods, 18(1), 100-106

Analysis software compatibility

InnoQuant delivers image files with a non-proprietary image format (multi-resolution TIFF) that opens up a world of possibilities for subsequent image analysis.

Non-exhaustive list of applications and third-part softwares operating with InnoQuant images:



Key user benefits

Fluorescence Cell and Tissue slide scanner



Quantitative data

Spatial signal homogeneity
Up to 16-bit TIFF images



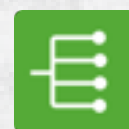
Reproducible results

Inherent hardware calibration
Argolight® validation slide



Homogeneous image

Point by point scanning
Full-width line scanning
No tiling, no stitching/shading



Throughput capabilities

24-slide autoloader
Multiple ROI per slide
Custom settings for each slide



Multiplexing capabilities

4 independent PMT
Up to 7 emission filters per channel



Remotely operable

Server based installation
User-friendly software interface



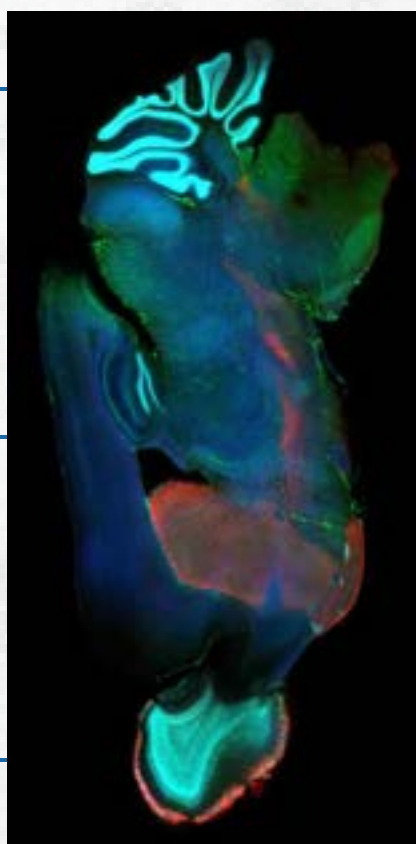
Quick time to results

4 colors simultaneously scanned
17 min for a 3.2 cm² area
No post-processing
Focus without user intervention

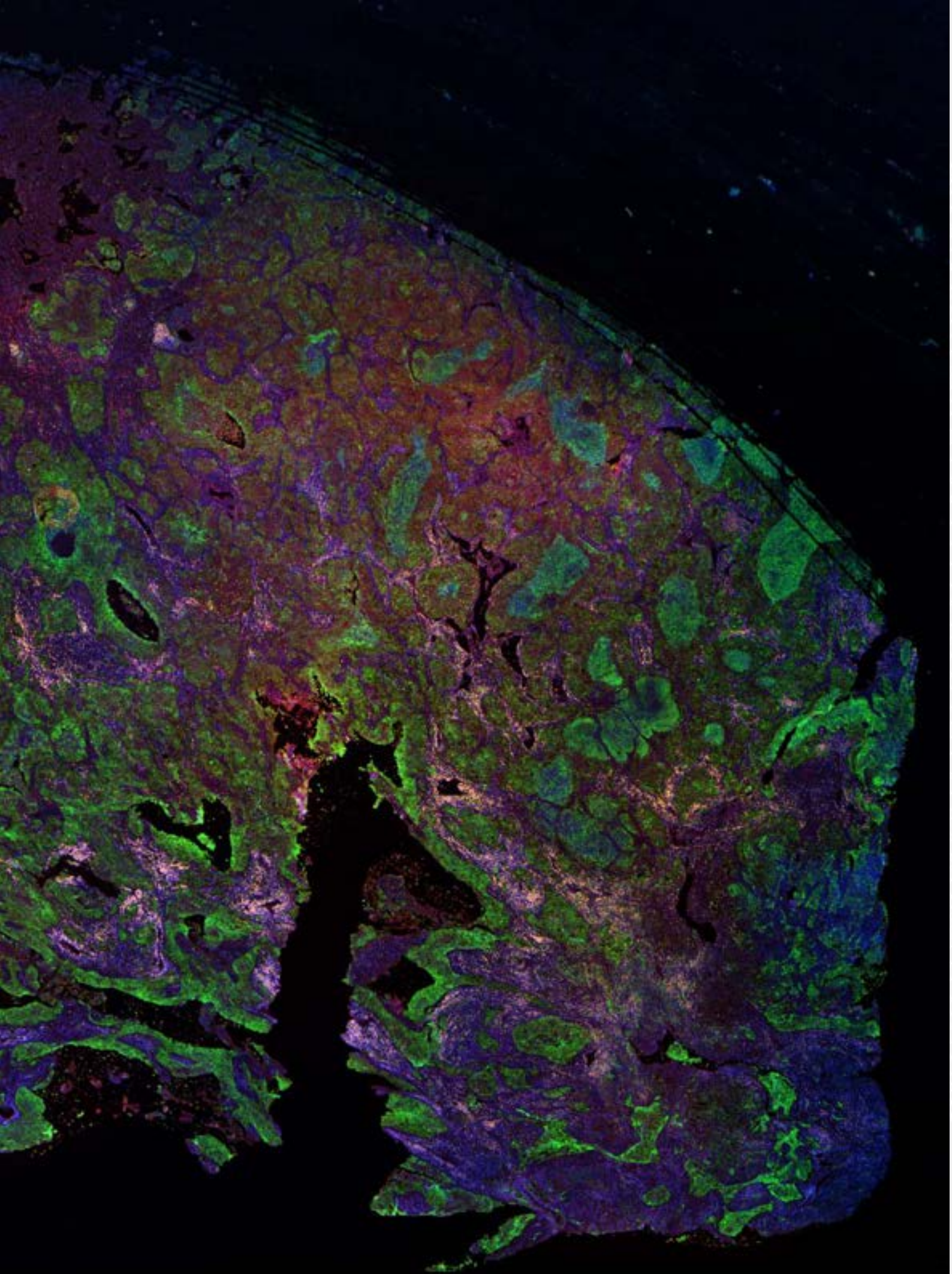


Easy image handling

Non-proprietary format
Multi-resolution TIFF
Compatible with multiple analysis softwares



FluoTissue®, RapiClear 1.52 prepared slide, Brain sample, 550 µm thick. SunJin Lab Co



Technical features

Key specifications

LASER EXCITATION WAVELENGTH	375 nm	488 nm	561 nm	640 nm
EMISSION FILTERS	FF01-440/40	FF01-513/17	FF01-593/40	FF01-680/42
	For customization: 6 additional position filters for each PMT			
COMPATIBLES FLUOROPHORES*	DAPI Alexa Fluor 350 Hoechst DyLight 350 Coumarin BV421 / BV510 BV603 / BV711 BV750 PacificBlue® CascadeBlue®	FITC Alexa Fluor 488 GFP Cy2 DyLight 488	TRITC Alexa Fluor 561 Cy3 DyLight 594 MitoTracker® Red	DRAQ 5 Alexa Fluor 647 Cy5 DyLight 650 MitoTracker® Deep Red
DETECTION TYPE	4 independant photomultipliers (PMT)			
PMT GAIN	Linear from 0,01 to 100%			
ACQUISITION MODE	4-color simultaneous acquisition			
FOCUS MODE	3 different focus options: autofocus, fixed focus and focus by content			
SLIDE SIZE	Compatible with standard microscopy slides: 26 x 76 mm / 1 x 3 inch Maximal standard thickness 1,8 mm / 0,07 inch			
LOADER CAPACITY	Single slide or 24-slide autoloader			
SCANNING AREA	Adjustable up to 26 x 76 mm / 1 x 3 inch			
SCANNING TIME	17 min for a scan area of 3.2 cm ² / 0.70 x 0.70 inch at pixel size 0.5 µm			
PIXEL SIZE	From 40 to 0.5 µm			
IMAGE FORMAT	Non-proprietary image format (multi-resolution TIFF)			
POWER SUPPLY	240 VAC, 1.2 A, 50-60 Hz			

*Non-exhaustive list of compatible dyes.

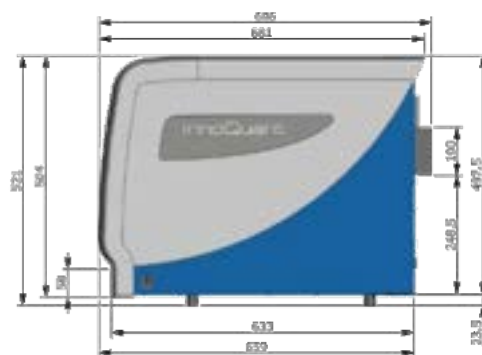
Size and weight



Single slide weight : 48 kg/105.8 lbs



Auto-loader weight : 52 kg/114.6 lbs



Class I laser product for research use only
May 2024. P_InnoQuant_rev01
Specifications subject to change without notice
Contact us for the most recent specifications

www.innopsys.com



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