

MIAS-2™ & eaZYX™ : Versatile Tools for Automated Multimode Microscopy Reading

MAIA SCIENTIFIC – Cipalstraat 3, 2440 Geel, Belgium
tel: +32 14 570 620, fax: +32 14 570 621, info@maia-scientific.com, www.maia-scientific.com



MIAS-2 is the first high throughput microscopic reader that combines 5 brightfield with up to 8 fluorescence modes, making it the most versatile multimode reader available today for cell-based assays, tissue and tissue sections, bead assays and small animal model organisms.

Besides a state-of-the-art CCD camera, MIAS-2 also features an intensified camera and proprietary object-based auto-focusing for real-time low-light observation below the sensitivity of the human eye.

Sub-visual observation enables to develop high information content screening (HICS) assays for targets with low expression levels (sensitive) or targets expressed under the control of weak gene promoters (physiological). Low-light observation can significantly cut HICS cost, by allowing to use lower assay reagent concentrations, and assay time by elimination of signal-amplification steps.

Reduced light input turns MIAS-2 into a non-invasive reader with low photo-bleaching or phototoxicity for time-lapse observations and frequent reanalysis of objects, which facilitates assay development.

Equipped with eaZYX imaging and automation software, MIAS-2 provides the capacity to work hands-on at the reader, to build your own applications or to apply powerful image analysis in custom built solutions. Over a mouse click you can switch to hands-off HTS mode. MIAS-2 brings automation from 'plate stack to database-ready result'. The time needed for assay result evaluation is drastically reduced by intuitive and easy-to-use 'on image' proofing tools.

To conclude, the flexibility, sensitivity and speed of MIAS-2 fit the future needs of assay development, assay optimization, high throughput screening and hit-to-lead projects. In terms of speed for money, MIAS-2 can compete with the fastest readers in the market.

High information content screening (HICS) is a widely interpreted concept. Minimalistically, HICS can be defined as screening campaigns in which 'more than one' endpoint is being recorded per assay entity. With microscopic readers, HICS became the process of automated image acquisition, image segmentation and the reduction of images to quantitative data of changes in cells.

The more ambitious view for HICS is a technology that allows reading and quantifying the complexity of biological phenomena. Observe signalling pathways in action. Watch proteins while being expressed and transported to their 'workplace'. Follow protein interactions inside organelles and cells. Quantify cell division, cell death, gene expression, cell motility, cell positioning in tissues, plants or animals. Ultimately, identify the modulating effects of drug candidates on the aforementioned events.

The trend from simple, single endpoint to complex multi-endpoint reading of a wider variety of features in cells or multi-cellular models at ever higher speed and sensitivity has lead to a need for microscopic readers with a different blend of features.

With MIAS-2, we have built a microscopic reader that brings you both speed and extreme sensitivity, in combination with the full flexibility of advanced research microscopes (Table 1, Figure 1).

Table 1. MIAS-2 readers Features Overview

Multimode reader

- Up to 5 brightfield and/or 8 fluorescent modes
- Up to 6 objectives (1.25x–63x)
- Images, super-images, plate overviews and video
- B/W, colour and ultra-sensitive intensified camera's
- Easy-to-use graphical interface

Flexibility

- Hands-on 'live mode' operation
- Hands-off operation to full automation
- 6-1536 well plates (SBS standards)
- Petri dishes, micro-arrays, micro slides, custom formats
- Sample acclimatization (optional)

Automation

- Robust object-based auto-focus, also in extreme low light
- Multimode readings of selected objects, wells, plates
- Multiple time-lapse video recordings in parallel
- 90, 210 or 300 plate capacity (optional)
- HTS backbone integration (optional)

Areas of applications

- Assay development, high information content screening, hit-to-lead work, kinetic and time-lapse reading

Assays and imaging applications

- Cells, tissues, arrays and beads
- Small animal model organisms, plant seeds

Sensitivity

MIAS-2 readers are equipped with state-of-the-art B/W or color cameras *plus* an ultra-sensitive intensified camera. The color and B/W cameras supports reading of signals of high to moderate and low intensity, typically created by today's assay technology. They also capture high-quality color and B/W images and video for documentation and images analysis purposes.

Intensified camera: extreme sensitivity, less phototoxicity

The MIAS-2 readers also feature an intensified camera for reading of very weak fluorescent signals (*weak-signal reading*) and/or observation with reduced light input (*low light input reading*). In combination with the robust, object-based auto-focus technology of the eaZYX software, the MIAS-2 readers will flawlessly focus on signals that are 'invisible to the eye' in the microscope and will bring them to life on the computer screen. In this way, the MIAS-2 reader provides a unique new avenue to access biology for low abundance targets. Alternatively, recombinant targets can be observed at physiological expression levels under the control of weaker gene promoter(s).

The capacity to read very weak signal with MIAS-2, allows the development of assays in which lower assay reagent concentrations are being used or in which signal amplification steps can be eliminated. As a result, the development of multi-label assays for multimode reading becomes much easier. Significant savings on assay cost and reductions in assay time will result from the use of the MIAS-2 reader.

The ability to observe with '*low light input*' is another advantage of the intensified camera. The MIAS-2 readers can read fluorescent signals of moderate or high intensity adequately with strongly reduced excitation light *input*. This feature reduces or even eliminates phototoxicity to living cells and small animal models organisms. Likewise, 'low light input reading' significantly increases the lifetime of fluorescent probes. In particular in long-term, multimode time lapse observation, 'low light input reading' is a very useful feature. To exploit the advantage of 'low light input reading' maximally, MIAS-2 readers are equipped with a filter wheel to reduce excitation light over a range of three decades.

'*Low light reading*' and '*weak signal reading*' apply to hands on observation and to HTS screening alike.

High resolution & high precision positioning

Multimode microscopy reading with MIAS-2 readers is fully supported for a range of six high-quality objectives, selected for their excellent resolution (high numerical aperture) and performant fluorescence characteristics. With the 40x objective, in-plane resolution is 0.35 micron (XY) and a focal depth (Z) of 0.89 micrometer. For ultimate flexibility and high quality image capture, three motorized intermediary magnifications allow perfect adjusting of the size of objects on the camera CCD chips that is optimal for image analysis (Nyquist sampling).

Ultra-precise object positioning and repositioning

The object positioning hardware of the MIAS-2 readers in

the X Y dimensions, in the plane of the object, and in Z, the focal plane, have a precision that largely exceeds the optical resolution of the reader. Ultra-precise object (re-)positioning allows revisiting wells or objects for capturing 'pixel-matched' images. This unique feature of MIAS-2 readers supports novel, flexible ways of image capture and advanced forms of time-lapse analysis which significantly speed up the process of multimode microscopy reading with the MIAS-2 readers.



Figure 1. The MIAS-2 readers.

Flexibility

Multimode microscopy reading

MIAS-2 readers feature supreme quality microscopy hardware with the highest level of motorization of the components in the fluorescence and brightfield light paths, all fully integrated with eaZYX software.

They allow automated image capture for up to a total of 13 different microscopy modes: five brightfield microscopy modes and four or eight fluorescent channels covering blue to deep red reading.

Depending on your R&D needs: ultra-sensitivity or high flexibility, we have designed two MIAS-2 readers. The MIAS-2 reader with *four* fluorescent modes has been optimized for sensitivity, the MIAS-2 reader with *eight* fluorescent modes aims at highest flexibility of reading across the visible spectrum. The brightfield and fluorescent modes of MIAS-2 readers will be customized to your assay needs.

Seamless capturing of large areas at high resolution

The eaZYX software features the *versaTILE technology*. It allows the user to 'zoom in' on objects by using high magnification and peak resolution, without losing the advantage of capturing large areas. For all six MIAS-2 objectives in combination intermediary magnifications, versaTILE technology will use the high-precision object positioning to shift the object exactly to create adjacent images that automatically match at their borders (figure 3a,b). The images composing an image tile are 'pixel matched'. Objects *on* the borders are precisely reconstituted in the image tile to allow unbiased image analysis of *all* objects in the image tile. The number of images in a tile is set by the user in function of the assay characteristics. In this way, multimode reading of biological assays can be easily adapted to the typical variations of cell-based assay

conditions. The user can freely combine a selected magnification with size of the image tile, according to statistical (e.g. object population size) and image resolution requirements of the assay. The versaTILE technology also features automated object edge detection for image capture of complete wells or tissue sections at variable positions on a microscope slide (figure 3b).

Capture multiple time-lapse video's in parallel

Pixel-matched revisiting of multiple positions in plates over time allows to create multiple time-lapse video's in parallel in a single MIAS-2 reader. The only limiting factor to the number of time-lapse recordings is the time *you* allow between image capture cycles.

In addition to the typical time-lapse settings, the embedded **versaTIME technology** of eaZYX software allows greater flexibility of time-lapse observations. The user can select the positions (or objects), set tile size for capture, read multiple consecutive modes, acquire still images or a create short real-time video sequence at each time-lapse interval. These advanced time-lapse features enable the almost complete automation of assay time point optimization with living cells or small model organisms in culture.

For observations of cellular events that are susceptible to the mechanical disturbance, the object positioning hardware features the option to set the acceleration and deceleration speeds of the object stage.

Wide range of object carriers & assay applications

HTS applications of MIAS-2 readers include cell-based assays, tissue-based assays, (behavioral) small model organism screens (zebra fish, *C. elegans*, *Drosophila*, *Arabidopsis*), bead assays, ADMET assays, *in vitro* and *in vivo*, with living and fixed objects (figure 2a-g).

MIAS-2 readers accept multi-well plates, Petri Dishes, micro-arrays and microscope slides placed in specific carriers fitting the 96-multiwell plate footprint. Likewise, MIAS-2 also reads tissues and tissue sections for histology and histopathology, blood smears, microarrays, ... from preclinical (transgenic) animal studies and/or clinical studies.

Speed

The unique blend of sensitivity, resolution, precision flexibility of MIAS-2 readers and the resulting new ways of microscopic reading, impact positively on the speed at which you can develop, execute and follow up HTS screening campaigns through the R&D value chain.

Real-time focus and image capture on very weak signals

Image capture of weak signals requires integration over time when done with standard cameras. The time delays associated with integration are unacceptable in high throughput high information content screening.

Two unique feature of MIAS-2 readers: the intensified camera combined with robust real-time object-based auto-focus supports real-time image capture on signals of extremely low intensity and high intensity and all the inherent advantages.

Speed up multimode reading

In addition to object based auto-focusing, eaZYX also supports reading at low resolution with preset focus planes according to plate type characteristics. This eliminates the need to focus if unnecessary and takes plate characteristics into account.

In high resolution reading, or weak-signal reading, when robust object-based auto-focusing is essential, the autofocusing is limited to the first reading cycle. The eaZYX software will store the XY positioning coordinates visited and the focal (Z) position while auto-focusing and capturing in the first reading cycle. These XYZ coordinates are used to revisit **exactly** the same XYZ positions in plates during all subsequent microscopy modes. As a result, the cycle time for second (and all subsequent) microscopy modes will be significantly reduced.

'Hands-on operation' to 300-plate screening campaign to HTS embedding. MIAS-2 readers conveniently allow 'hand-on observation' of brightfield, fluorescent as well as sub-visual signals. Automation options of MIAS-2 readers include 96-, 210- or 300-plate stacking robotics in stand alone set up. Embedding in a wide range of HTS robotics is supported using robust client server software.

Ultimate flexibility with MIAS-2 & eaZYX

Capture information the way you want. MIAS-2 and eaZYX automation allow you to combine *all* microscopy modes, with *all* acquisition modes (image, tiled image, mosaic image, video or time-lapse video) at the level of one object, selected objects, wells, plate, plate stack or batch of plates.

Open image-analysis work environment. You can batch-export images and videos from the MIAS-2 reader to your preferred image analysis work environment. Likewise, you can batch-import images and videos to apply eaZYX imaging powerful analysis tools based on 'Scale Space' (ter Haar Romeny *et al.*, 2001; Van Osta *et al.*, 2002a).

Move fast through your HTS queue. eaZYX solutions are cost-cutting tools for 'plate stack to database-ready result' automation, that can be applied to all microscopic and image acquisition modes at a wide range of magnifications. Changes in your assay technology can be implemented without the need for new software.

Easy integration in HTS backbones. The modular architecture of the eaZYX software supports embedding of MIAS-2 in a wide range of HTS automation platforms.

Table 2. MIAS-2 readers: Specifications at a glance

Multimode brightfield

- 100 W halogen light source
- 5 brightfield microscopy modes (darkfield, phase contrast, DIC contrast, ...)
- Used in combination with fluorescence modes

Multimode fluorescence

- 75 W Xenon light source
- 300-800 nm excitation range
- Attenuation of excitation with 5 neutral density filters for living cell applications
- Standard 4 fluorescence modes (DAPI – FITC/GFP – YFP – TRITC/RFP)
- Upgrade to 8 fluorescence modes (optional)

Object positioning accuracy & features

- Accurate pixel-matched revisits of wells for multimode and kinetics readings
 - X and Y (stage): <0.30 micron
 - Z (focus): <0.10 micron
- Controlled stage acceleration and deceleration for gentle positioning of e.g. living cells

Optics

- 5 objectives standard 2.5x - 40x (6th optional)
 - option 1.25x or 63x extension
- Resolution (at 40x, numerical aperture=0.75):
 - XY: 0.35 micron
 - Z (focal depth): 0.89 micron
- Optics to optimize object size on CCD chip

Camera's

- 2 cameras, with automated switching
- Colour camera for brightfield and fluorescence
- Intensified camera for low light fluorescence
- Other camera combinations optional

Sample handling

- Compatible to SBS microplate standard
- Integrated barcode reader
- Integrated robotic plate station (optional)
 - Capacity 90, 210 or 300 plates
- Incubation chamber inside reader (optional)

eaZYX-software: your assistant for MIAS-2

eaZYX is an 'easy-to-use' software for image acquisition and image analysis. eaZYX operates the MIAS-2 like a hands-on research microscope or for a 300 plate HICS campaign from 'plate stack to database-ready' result. 'Easy' also means you can switch from hands-on to automated mode with a mouse click.

eaZYX-AUTOMATION drives both the MIAS-2 reader and the robotic plate station. It controls all settings for the brightfield and fluorescence image acquisition modes. It runs an object-based auto-focusing of unseen speed and robustness even in extreme low-light conditions (Geusebroek *et al.*, 2001). It schedules the different selected microscopy modes for the acquisition of images, tiled images, video and/or time-lapse movies per object, well, plate or batch of plates. Captured images, image tiles and video (figure 3) are stored in their relational multi-mode and meta- context. eaZYX automation's modular software architecture facilitates connecting MIAS-2 to scheduling and control software of robotic HTS backbones.

eaZYX-IMAGING is the engine that provides a hands-on image segmentation work environment and high-end image analysis (see text box below). eaZYX imaging detects and quantifies the object's characteristics. Detailed result data tables and descriptive statistics summaries (e.g. per well) are produced (figure 3d). Images and/or data files can be imported into spreadsheet programs, bio-statistical software, bio-informatics-, drug discovery- or image databases.

eaZYX-PROOFING is a set of easy-to-use, on-image proofing tools for the researcher to review large sets of assay results quickly. They conveniently relate the output data tables directly to objects in images and vice-versa (figure 3c,d).

eaZYX-SOLUTIONS are add-on protocols, specifically designed to develop and run high throughput, high information content screening campaigns. They incorporate all the functions needed to swiftly optimize assay conditions in assay development, while controlling assay time and cost in screening (figure 3d).

eaZYX-IMAGING: computer vision that matches human vision

eaZYX-IMAGING uses a holistic principle to 'look' at images. Unlike many image analysis softwares, it does not identify objects by applying a 'pixel-by-pixel' image segmentation to compose the objects in image. eaZYX applies the principles of 'Scale Space' theory to microscopic images (Van Osta *et al.*, 2002). The *entire image content is used in the object identification process*: the pixel features plus their 'morphological' appearance in the image. To identify groups of pixels with recurrent morphological features (objects), eaZYX-IMAGING queries images for shape criteria, spectral and intensity characteristics in a one-pass procedure.

These holistic query tools were used to create the patented object-based auto-focusing tools of the MIAS-2 readers (Geusebroek, 2000) and of the applications shown in figure 2. They also improve the quality of object identification, with major improvements in conditions of variable backgrounds – such as e.g. in images of multi-well plates captured in brightfield microscopy modes – and in high-noise images, such as in real-time auto-focusing and image capturing of very weak fluorescent signals with the intensified camera. In the latter case, no noise reduction is needed to identify objects in images.

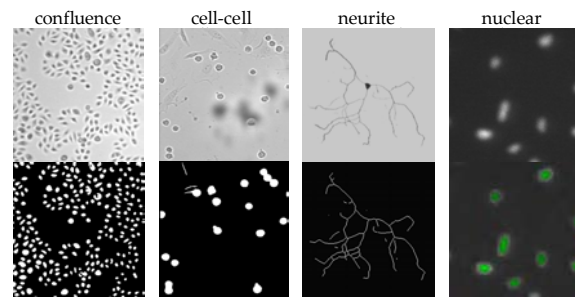
Table 3. MIAS-2 & eaZYX assays & applications

	1*	2*	3*
<i>Cell-based assays:</i>			
• Confluence measurement (Figure 2)	⊗	U	I
• Adherent cell growth	⊗	U	I
• Cell migration	⊗	U	T
• Cell motility	⊗	U	T
• Cell-cell adhesion to monolayer (Figure 2)	⊗	U	I
• Neurite outgrowth (Figure 2)	⊗	U	I/T
• Nuclear translocation (Figure 2)	⊗	Y/P	I
• Protein co-localization	⊗	Y/R	I
<i>Weak gene promoters:</i>			
• Transcription factors (Figure 2)	⊗	R	I
• Nuclear lamins (Figure 2)	⊗	R	I
• Low level protein expression (Figure 2)	⊗	R	I
• Protein localization (Figure 2)	⊗	R	I/V/T
• Receptor internalization	⊗	Y	I
• Apoptosis	⊗	Y/P	I/T
• Oocyte maturation <i>in vitro</i>	⊗	U	I
• Computer-aided cell harvesting	⊗	U	I
<i>Bead assays:</i>			
• Bead-binding assays (fig)	⊗	P	I
• Cells on beads	⊗	U	I
<i>Tissue and tissue sections:</i>			
• Skin: granular layer detection (Figure 2)	⊗	H	I
• Apoptosis (DAB brown)	⊗	H	G
• Goblet cell counts (Figure 2)	⊗	H	I
• Mucus production (Figure 2)	⊗	H	I
• Nerve (de-)myelinisation (Figure 2)	⊗	H	I
• Leukaemia cell counts in blood smears	⊗	H	G
• Hibernating/infarcted myocard (fig. 2)	⊗	H	I
• Tissue/tumour vascularisation (DAB)	⊗	H	G
• (tumour) cell proliferation (Figure 2)	⊗	Y/W	G
<i>Model organisms:</i>			
<i>C. elegans:</i>			
• Population density (biomass, Figure 2)	⊗	U	I
• Population composition (stages)	⊗	U	I
• <i>egl</i> or multi-vulva assay	⊗	U	I
• Muscle positioning (Figure 2)	⊗	U	I
• Motion (liquid culture, Figure 2)	⊗	U	T
• Motion (on agar, Figure 2)	⊗	U	V
• Compound toxicity (LD ₅₀)	⊗	U	T
• Embryonic development	⊗	U	I
• Pharynx pumping	⊗	UR	I
<i>Zebra fish:</i>			
• Angiograph / angiogenesis (Figure 2)	⊗	P/Y	I
<i>Arabidopsis:</i>			
• Seed viability	⊗	U	I

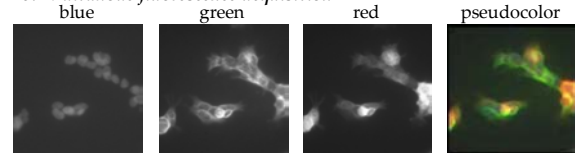
(*) 1. *microscopy modes*: B: brightfield; F: fluorescent;
 2. *assay type*: Y: immuno-fluorescence; W: immuno-cytochemistry;
 R: reporter proteins; P: fluorescent probe; H: histochemistry;
 U: unstained;
 3. *acquisition mode*: I: images; G: gigapixel tiled images; T: time lapse;
 V: real time video

Figure 2 (to the right). Examples of MIAS-2 and eaZYX image acquisition and analysis.

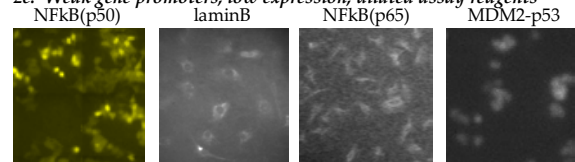
2a. Brightfield & fluorescent multimode reading in cell-based assays



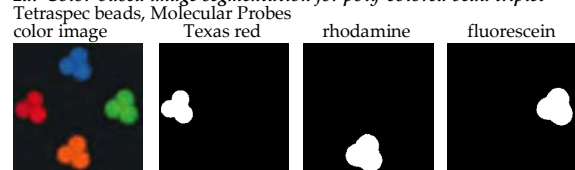
2b. Multimode fluorescence acquisition



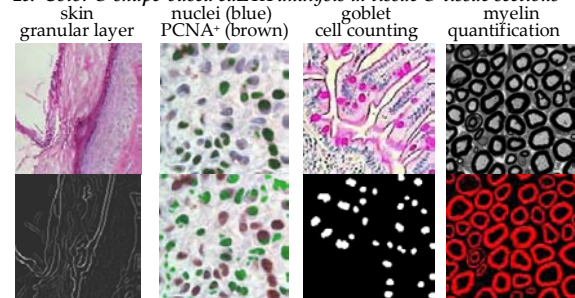
2c. Weak gene promoters, low expression, diluted assay reagents



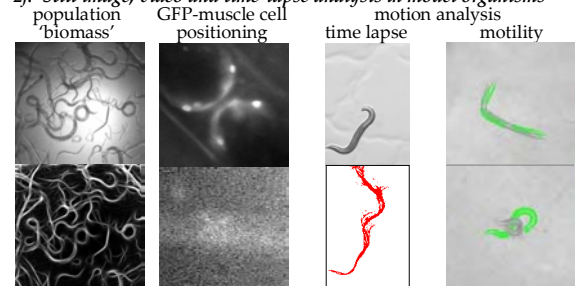
2d. Color-based image segmentation for poly-colored bead triplet



2e. Color & shape-based eaZYX analysis in tissue & tissue sections



2f. Still image, video and time-lapse analysis in model organisms



2g. Zebra fish angiograph

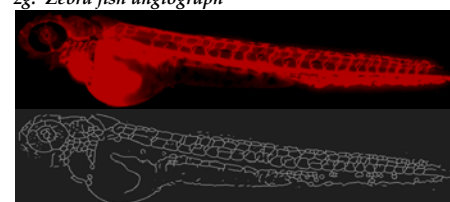


Table 4. Facilitating eaZYX features

XY object positioning

- Pixel-matched revisiting of XY positions
- Export of XY coordinates of objects in plates

Robust real-time auto-focusing (Z positioning)

- Complete autofocus in 300 milliseconds
- Proprietary object-based auto-focus with robust performance in low light / high resolution conditions
- Auto-focus on sample objects with fixed and/or variable offset from focus found
- Re-focus pattern matching plate bottom quality

Image & video capture rates

- 40ms (PAL), 33ms (NTSC)

Super-image capture (image tiles)

- Multiple fields per well (pixel matched or free)
- Pixel-matched image tiles (68 Gigapixel super image)
- Automated edge detection sections
- Tiled overviews of plates

Time-lapse image and video capture

- Parallel time lapse recording on multiple positions (with pixel matched revisiting of each position)
- Capture video at pre-set time-lapse intervals

Image import and export

- Import: TIFF (B/W & Colour), JPEG (B/W)
- Export: TIFF, JPEG, BMP and GIF
- Video in QuickTime™ format.
- Individual images (for documentation or publication)
- Batch export (per well, plate or plate batch)

Acknowledgement

We are grateful for the use of samples and images provided by many of our colleagues at various academic and company customer sites.

References

Geusebroek JM,. Robust auto-focusing in microscopy, 2000, Cytometry, **36**, 1-9 & patent WO 0075709

ter Haar Romeny BM, Color differential structure, 2001, In: Lecture Notes in Computer Science 2106, Eds: Springer Verlag, pp. 353-361, ISBN 3-540-42317-6

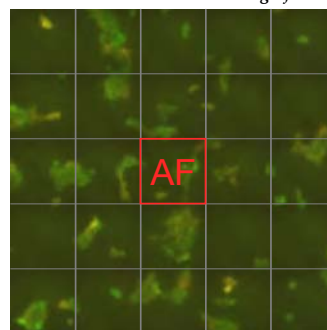
Van Osta P, The principles of scale space applied to structure and colour in light microscopy, 2002a, Proc. Royal Microsc. Soc., **37**: 161-166

Van Osta P, Enhanced One-step Nematode Recognition on micrographs of Living *C. elegans* Cultures in 384-well Plates using Linear Scale Space Mathematics, 2002b, Poster at the European Worm Meeting

Ver Donck K, High density *C. elegans* screening: an automated phenotype analysis platform, applied to the Unc-53 (Steerin) pathway, 2000, Poster at the European Worm meeting

Figure 3 (to the right). Illustration of pixel-matched image acquisition, click & zoom evaluation (Ver Donck et al., 2000), mosaic images for plate overviews (Van Osta et al., 2002a) and on-image proofing of image analysis results.

3a. Pixel-matched tiled scanning of three fluorescent modes



25 image tiles scanned 'spiral out' around one auto-focus point (AF)

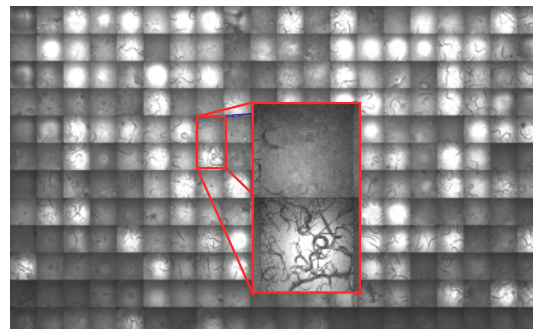
3b. 'Unlimited' tiled scanning of wells & sections with multiple focus

40x objective (dry)
473 image tile / well
20x focus / well
28380 images / plate
0.5 seconds / image
4 hours / plate
12800 x 12800 pixels

63x, oil
1300 images
433x auto focus
40 minutes scan
51x66 = 3366 tiles
26112 x 33792 pixels
Automated edge detection

Click & zoom from tile to high resolution images for evaluation

3c. Mosaic image of a 384 well plate with click & zoom function



3d. On-image proofing for quick evaluation of screening results

Object	Area	Perimeter	Contour ratio	Grey mean
1	433	89.66	1.22	189.85
12 *	660	94.08	1.03	170.7
13 *	99	35.34	1	164.25
14	228	54.34	1.02	194.89
15	448	75.81	1.01	208.04

