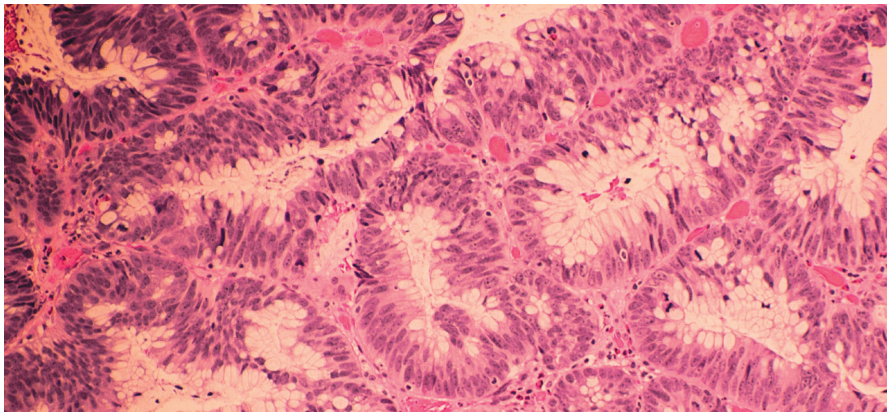
High Spatial resolution FTIR imaging of biomedical tissue samples using a novel method of magnification enhancement

Application note

Biomedical Research



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Introduction

Fourier Transform Infrared (FTIR) imaging is a well-established analytical method for obtaining spectral and spatial information simultaneously in the micron-size domain. Here the technique has been applied a to biomedical research. Over recent years, interest has increased in pushing the diffraction limited spatial resolution performance of FTIR imaging systems, primarily using synchrotron based systems.

In this application note, we present a novel method of magnifi cation enhancement achieved using existing objectives. The result is an FTIR system with high spatial resolution imaging capabilities in the order of

1 µm/pixel. Uniquely, this configuration conserves the relatively large working distance of regular objectives (~21 mm) by not requiring an objective change between magnification settings.

In order to assess and compare the performance of the new method, FTIR images of a biomedical sample (unfixed, cryosectioned mouse brain) were obtained using standard magnification FTIR, high magnification FTIR and multi-beam synchrotron-based FTIR [1].

Experimental

Instrumentation

An Agilent Technologies Cary 670 FTIR spectrometer, coupled to a Cary 620 FTIR microscope fitted with a high energy globar light source, was used to acquire data under standard magnification and high magnification conditions. For comparison purposes, data was also collected using a non-Agilent FTIR imaging system coupled to a multi-beam synchrotron (IRENI) source [1].

Operating parameters for each technique are given in Table 1.

Biomedical sample

A 7 µm thick cryosection taken from a 10 month old triple transgenic (3xTg) mouse brain was mounted on a BaF_2 window. 3xTg mice carry specific genetic mutations that lead to the formation of amyloid plaques in the brain, characteristic of Alzheimer's Disease, (Table 2). Protocols for sacrifice followed the guidelines established by the Canadian Council for Animal Care, which have been approved by Management Review committees at the University of Manitoba and University of Toronto.

Table 2. Mutations in the 3xTg mouse model

Swedish mutation (K670N/M671L) in APP
Presenilin mutation PS1(M146V)
Human four-repeat Tau mutation P130

Table 1. Instrument operating conditions

Parameter	Thermal Source Laboratory Standard Magnification	IRENI: Multiple Beam Synchrotron Radiation Source [1]	Thermal Source Laboratory High Magnification	
FTIR spectrometer	Agilent Cary 670	Non-Agilent spectrometer	Agilent Cary 670	
FTIR microscope	Agilent Cary 620	Non-Agilent microscope	Agilent Cary 620 (64x64)	Agilent Cary 620 (128x128)
MCT FPA detector (pixel)	64×64	64×64	64x64	128x128
Objective	15x, 0.5 NA, 24 mm working distance	74x, 0.65 NA, 1 mm working distance	15x, 0.62 NA, 21 mm working distance Further automated 5x magnification enhancement provided within microscope	15x, 0.62 NA, 21 mm working distance Further automated 5x magnification enhancement provided within microscope
Pixel size (µm/pixel)	5.5	0.54	1.1	1.1
Single Tile FOV (µm)	350x350	35x35	70x70	140x140
Source	Standard, High Energy, Globar Mid-IR Light Source	12 Beam Synchrotron Radiation Source [1]	Standard, High Energy, Globar Mid-IR Light Source	Standard, High Energy, Globar Mid-IR Light Source
Scans	128	256	256	256
Spectral Res (cm^{-1})	4	4	4	4
Tiles (Area)	1 (350x350 µm)	4x3 (138x104 µm)	3x3 mosaic (210x210 µm)	Single tile (140x140 µm)
Total time (mins)	2	72	40	6
Time per tile (mins)	2	6	4	6

Results and discussion

The photos and chemical images of Figure 1 A-C clearly show the abundance of important components in a 3xTG AD mouse brain. The images were obtained from the standard Agilent microscope configuration, the synchrotron (IRENI) and the Agilent high magnification configuration.

The results show that the new Agilent high resolution technique provides significantly increased spatial resolution, with a pixel size of 1.1 µm/pixel compared to 5.5 µm/pixel obtained with the standard Agilent microscope configuration. The new modification provides equivalent, if not improved results, compared to those obtained using IRENI, the multi-beam synchrotron source with wide-field illumination and effective geometric pixel resolution at or beyond the diffraction limit across the entire spectrum.

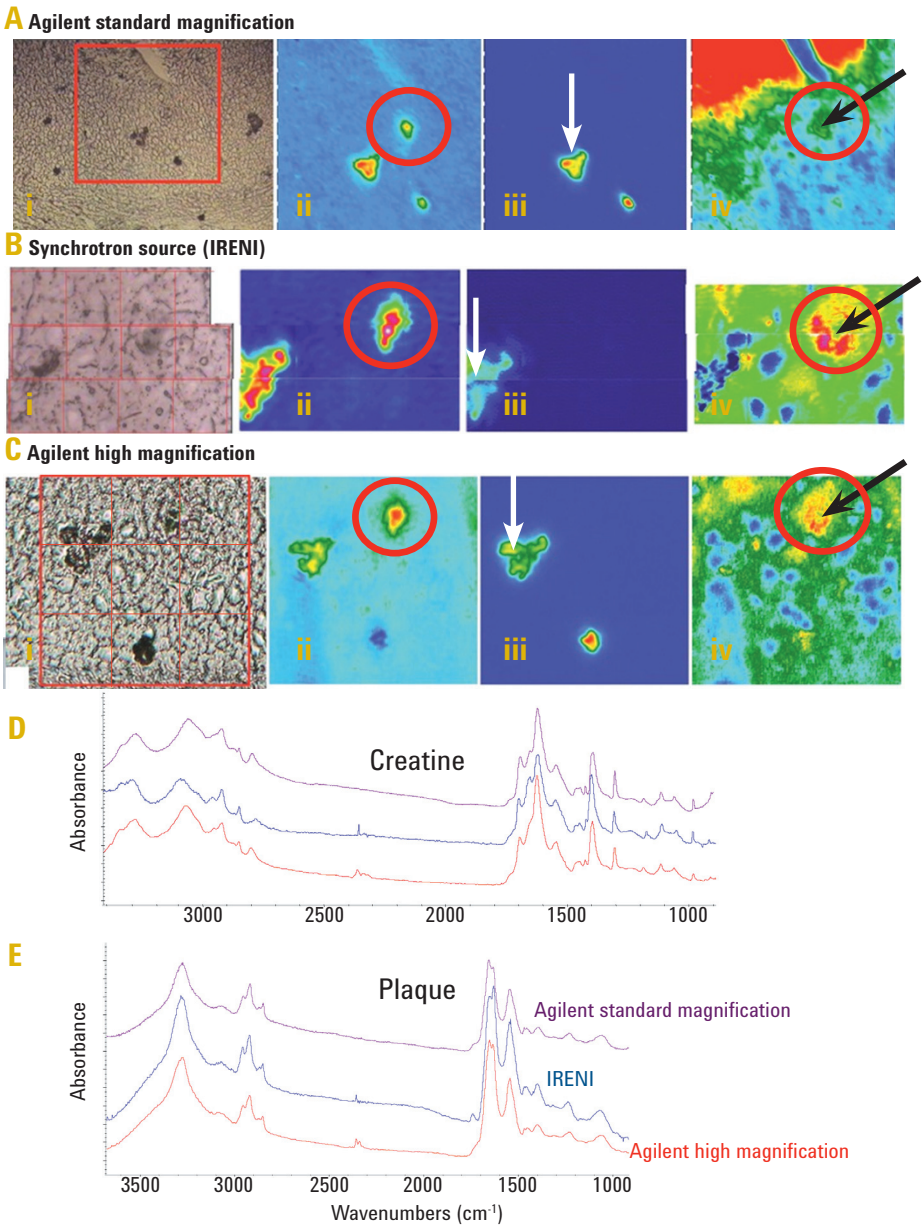

Figure 1. Excellent spectral quality from each of the 3 instruments. A, B and C: (i) visible images and chemical images of 3xTg mouse hippocampus processed for ii) Plaque and creatine (1630 cm^{-1} band) iii) Creatine only (1305 cm^{-1} band) iv) Lipid (2850 cm^{-1} band). Black and white arrows point to senile plaque and creatine deposits, respectively. D and E: Spectra from (D) Creatine crystalline deposit (E) Plaque core, from each instrument (color coded text and spectra). Heavy line red circles show location of the only plaque in this sample. See further comments next page. NOTE: Measurements were made approximately 12 months apart. While both spectra are similar, showing excellent S/N and spectral resolution, there are subtle differences that can be noticed. These are due to oxidation of the sample over this 12 month period.

The increased spatial and spectral detail (through having less pixel-to-pixel blurring) is invaluable when working with micron scale samples, as information is revealed that cannot be resolved using a standard microscope configuration.

With a pixel resolution of 5.5 µm/pixel, the spectrum is the sum of all chemical components lying within the sampled pixel area. Upon increasing the effective magnification using the novel automated and software controlled "high magnification mechanism", and importantly, without changing the objective and thus preserving the full 21mm objective working distance, pixel resolution to 1.1 µm/pixel is achieved.

In the example provided in Figure 2, the inclusion highlighted in the image with the arrow is revealed as a physically smaller, more concentrated object which would otherwise be lost within the larger pixel. It is important to note that along with improved spatial resolution, comes a more "pure" FTIR spectrum, as demonstrated by the stronger CH bands of the lipid inclusion in high magnification mode, that are otherwise averaged out in the standard magnification mode. Comparison spectra are shown in Figure 2.

Figure 3 shows an image of a dense plaque core (A) that is surrounded and infiltrated with lipid-like components (B) [2]. This figure shows that the same level of detail can be obtained using an high magnification instrument as with IRENI (see Figure 1 B iv), as would be expected for this wavelength region.

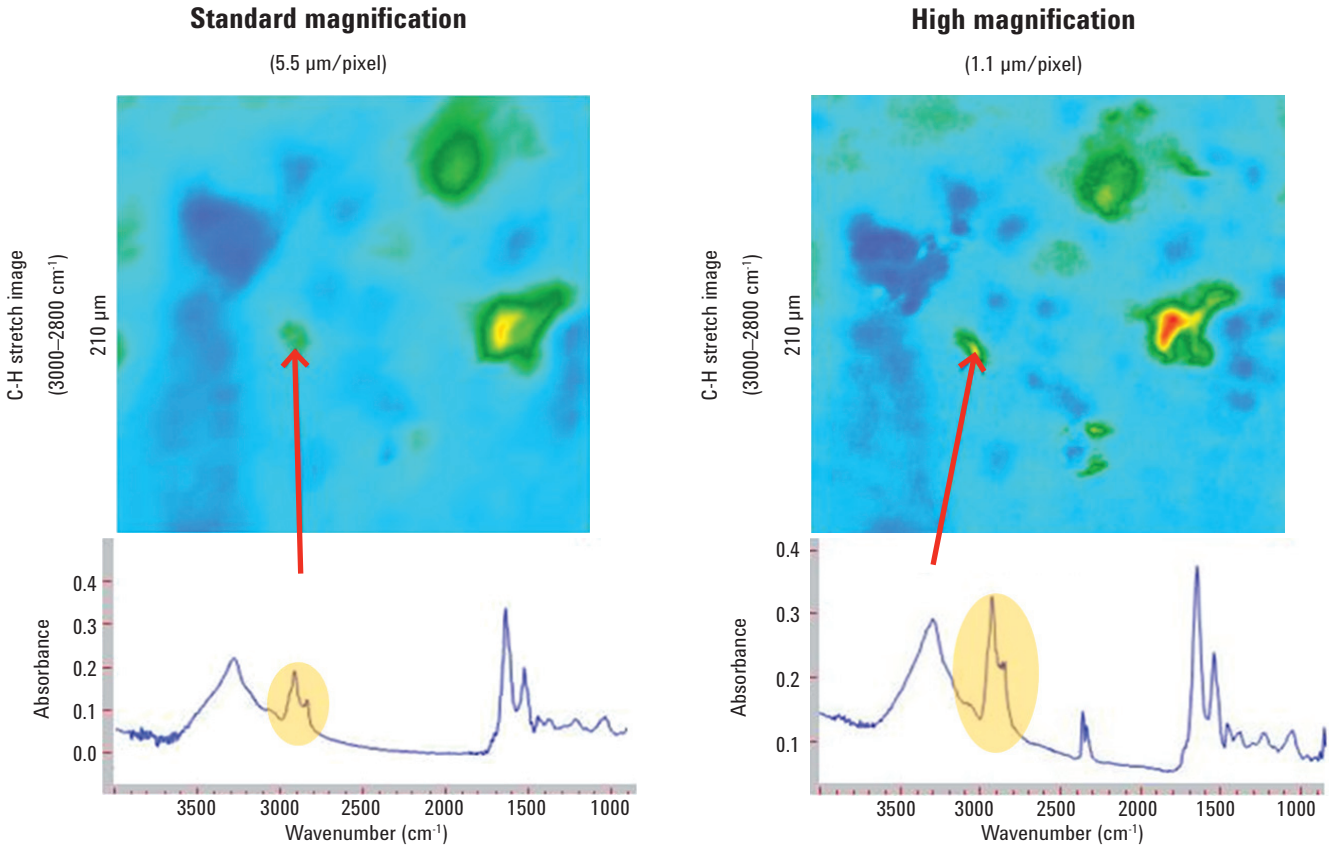

Figure 2. Comparison of standard and high magnification Agilent microscope. Processed false color images generated by integration of CH stretch bands. Left image shows portion of the standard, single low-resolution tile, cropped and zoomed to match the high magnification image. Spectra taken from same spot (red arrows) in each image; shaded region highlights CH stretch.

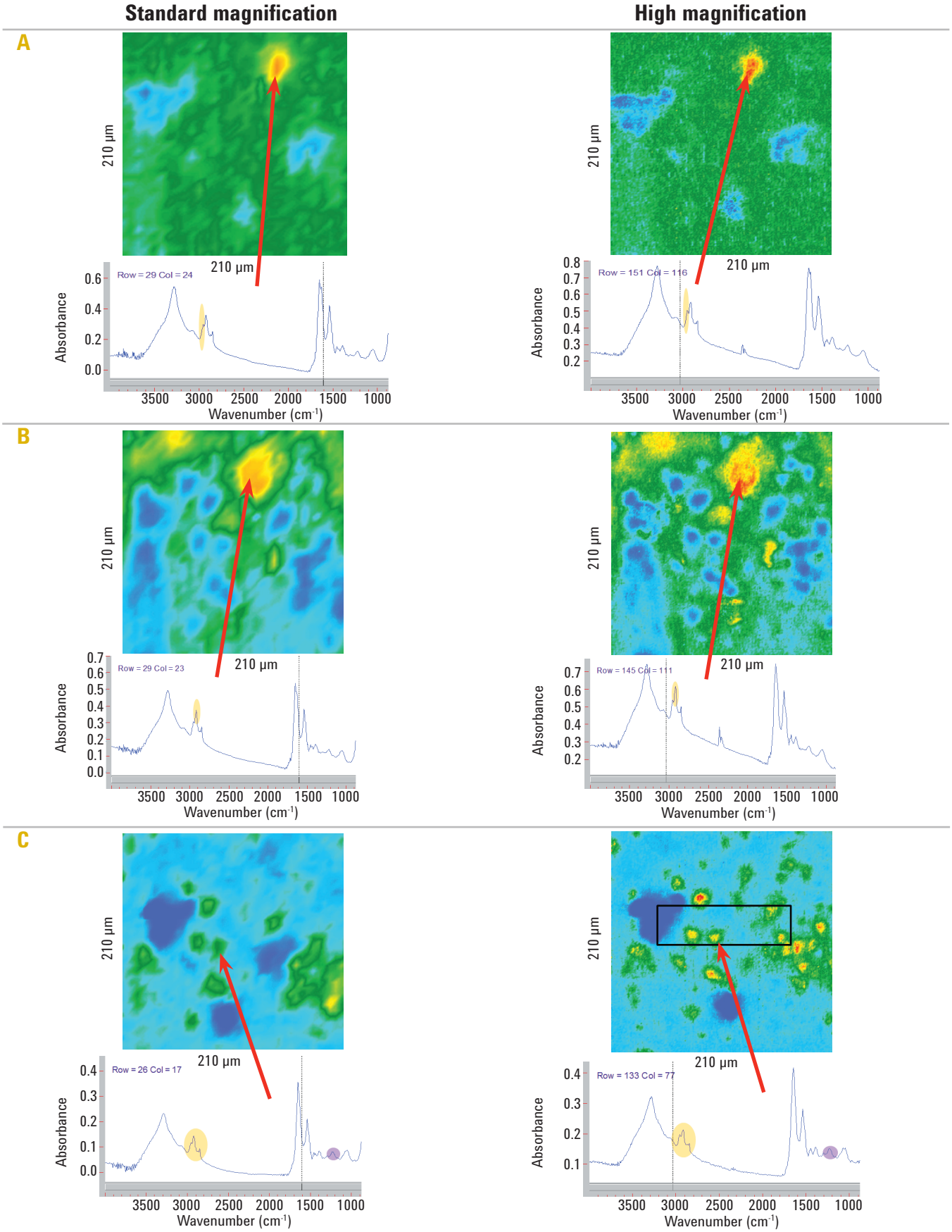

Figure 3. Comparison of standard and high magnification Agilent imaging systems. Processed false-color images from Figure 2, for A) Plaque core band; B) Asymmetric CH_2 band; and C) Ratio of phosphate (shaded purple) to symmetric CH_2 bands. Note: The black box on the high magnification "C" image denotes the cropped area displayed in Fig 4B.

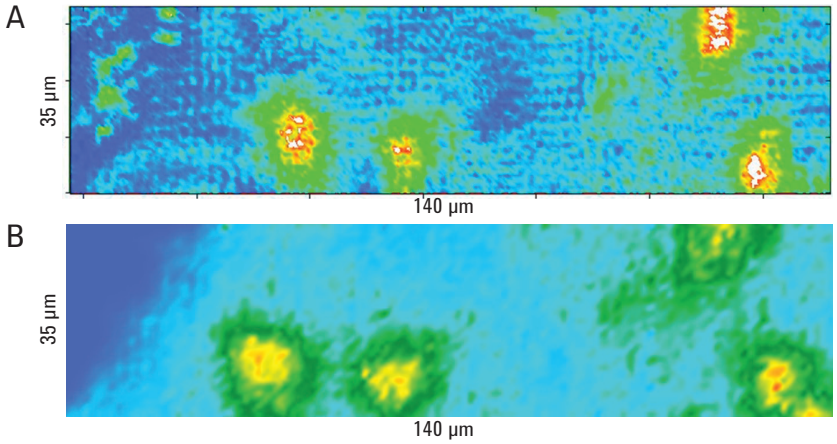

Figure 4. A) Low wavenumber imaging (as per processing of Fig 3C), applied to the bottom 1x4 rows of tiles from IRENI data (Figure 1B iv) to demonstrate the low wavenumber artefacts B) For comparison, the same cropped region from Fig 3C from the Agilent high magnification globar thermal source FTIR imaging, demonstrating no such artefacts.

Images reliant on longer wavelength bands sometimes include artefacts with the present IRENI system, possibly owing to some baseline fluctuations from the beams (Figure 4), which are not present in the high energy globar source of the Agilent FTIR imaging system as evident by the lack of any speckle-like pattern in the images of Figure 3C.

Conclusions

The results demonstrate that compared to standard magnification (5.5 µm), operating in high mode mode (1.1 µm), offers significantly added spectral and spatial detail and compared to synchrotron based high magnification systems, equivalent or better detail is observed, especially in the information rich fingerprint region, below 1800 cm^{-1}.

Additionally, image acquisition times are ~10x faster than the gold-standard multi-beam synchrotron instrument owing to the significantly larger field of views, together with the fact that full sized (128x128) FPAs can be used.

Furthermore, the ability to operate in standard magnification (5.5 µm) and high magnification (1.1 µm) modes without changing the objective, thus preserving the full objective working distance of 21 mm, means that users are not limited in the sample shape, size and form that can placed and measured beneath the microscope objective.

References

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