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IR microscopy utilizing intense supercontinuum light source

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Abstract: Combining the molecular specificity of the infrared spectral region with high resolution microscopy has been pursued by researchers for decades. Here we demonstrate infrared supercontinuum radiated from an optical fiber as a promising new light source for infrared micro-spectroscopy. The supercontinuum light source has a high brightness and spans the infrared region from 1400 nm to 4000 nm. This combination allows contact free high resolution hyper spectral infrared microscopy. The microscope is demonstrated by imaging an oil/water sample with 20 μm resolution.

OCIS codes: (180.0180) Microscopy; (190.4370) Nonlinear optics, fibers; (320.6629) Supercontinuum generation; (110.2350) Fiber optics imaging.

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Introduction

Infrared microscopy is utilized in a wealth of applications ranging from forensic science and cellular biology to sustainable energy research [1, 2], but the development and accessibility of infrared microscopy is severely limited by the absence of a bright and spatially coherent broadband infrared (IR) light source. Conventional infrared microscopes are based either on incoherent thermal IR sources with very low brightness, or complex sources based on synchrotron radiation [3, 4]. Here we demonstrate a broad band all fiber-based source of infrared radiation having the brightness of the synchrotron, and for the first time to our knowledge we demonstrate high resolution contact free hyper spectral infrared microscopy utilizing such an IR supercontinuum source.

The infrared source is based on supercontinuum generation in an optical fiber. Supercontinuum (SC) [5] generation in the visible spectral range has led to several breakthroughs in spectroscopy and imaging, most notably the development of the ultra-precise frequency comb used in optical metrology [6, 7], but the broad spectrum has also been utilized in microscopy [8, 9]. Recently, the supercontinuum technique was extended to the infrared spectral range beyond 2.6 μm by applying soft glass fibers such af ZBLAN [10, 11]. This provides many possibilities for applications in molecular spectroscopy [12]. SC generation is based upon nonlinear interaction between a short laser pulse and the fiber material in a process where the spectral width of the laser pulse widens from a few nanometers to several optical octaves [13–15]. The spectral brightness of the infrared SC is several orders of magnitude higher than that from a thermal infrared source and comparable to the brightness of synchrotron sources [2, 12, 16]. Since the SC is generated in a single spatial mode in the fiber, the light has a high degree of spatial coherence and is able to be focused to a diffraction limited spot size in principle.

In IR microscopy transmitted or reflected infrared light from a small sample area is spectrally dispersed and detected. Hereby an absorption spectrum for the area is recorded. Raster scanning the sample in two dimensions gives spectral information for the entire sample and a hyper spectral image is obtained [1]. Alternatively, the spatial information can be recorded with

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a focal plane array detector (FPA) [17]. In this way the IR microscope combines the molecular specificity of infrared spectroscopy with the spatial resolution of a microscope [2]. Hyper spectral IR microscopes are commercially available and have already proven to be a versatile tool in numerous areas [1–3].

The spatial resolution of IR-microscopes, based on incoherent thermal sources, is determined by a limiting aperture placed in front of the source. High spatial resolution measurements require small apertures and drastically reduce the amount of light available for imaging. This results in long acquisition times [18] and calls for a more intense and spatially coherent light source such as synchrotron radiation. The synchrotron sources are in many ways ideal for infrared microscopy [3, 18], but price, complexity, and availability seriously limits the spreading of IR microscopes based on such sources. A more widespread method for increasing the spatial resolution is based on the attenuated total internal reflection technique [19]. This works well for solid samples but in, for example, imaging of biological materials and cellular materials embedded in liquids it is not applicable and a contact free IR microscope is required.

In the following we utilize IR SC as a light source for contact free IR microscopy. As mentioned, the IR SC source has many of the same qualities as synchrotron radiation and therefore facilitate high resolution measurements. The IR SC light source can be applied in both a raster scanning and a FPA approach, but here we focus on demonstrating the raster scanning method.

Experimental setup

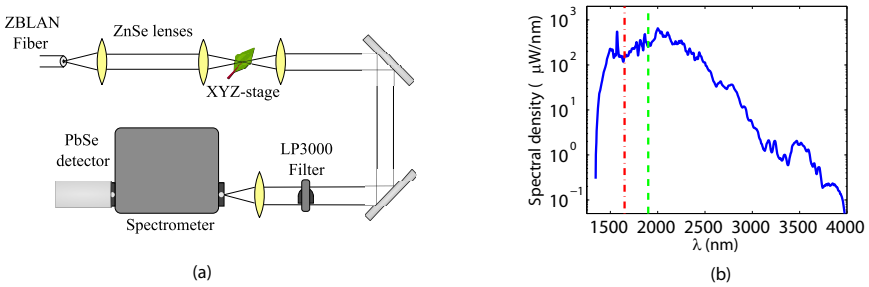

Fig. 1. (a) The IR microscope: The bright IR supercontinuum source is focused onto the sample. An image is obtained by raster scanning the sample and recording a transmission spectrum at each point. (b) The spectral output from the ZBLAN fiber. The red line indicates the wavelength of the ps pump laser and the blue line the ZDW of the fiber.

A schematic overview of the microscope setup is seen in Fig. 1a. The light source is 10 m of step index ZBLAN fluoride fiber pumped by a 40 MHz fiber-laser emitting 1 ps pulses at 1900 nm with a pulse energy of 16 nJ. The ZBLAN fiber has a core diameter of 7.0 μm and a numerical aperture NA = 0.2. The fiber is single mode for wavelengths longer than 1.8 μm and the zero dispersion wavelength (ZDW) is approximate 1.6 μm. As shown in Fig. 1b, the generated SC ranges from 1.4 μm to 4.0 μm. The spectral density at 3.5 μm corresponds to a spectral brightness of 300 kW/(nm m^2 sr), which is of the same order of magnitude as synchrotron sources used for IR microscopy [18, 20].

The SC output from the fiber is collimated with an aspherical ZnSe lens, having a focal length of 6 mm and NA = 0.25. The collimated light is focused and recollimated using two lenses identical to the first lens. The focus between the two lenses determines the resolution of the microscope. The sample under investigation is sandwiched between two 2 mm thick CaF_2 windows. A spacer between windows defines the sample thickness to $\sim$25 μm. The sample is

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placed in the focus and its position can be controlled with 1 μm precision by a motorized XYZ stage. The light transmitted through the sample is focused at the entrance slit of a monochromator by a 15 cm focal length ZnSe lens. The monochromator has a spectral resolution of 2 nm and second order artifacts are avoided by a 3000 nm long pass filter. The signal is measured with a PbSe detector. The sample is raster scanned in two dimensions at a single wavelength such that the relative transmission at this wavelength is obtained for a grid of the sample. In each point of the grid the measured signal is averaged over 150 ms for better signal to noise ratio. This procedure gives an image showing the absorption in the sample. If a relative absorbance image is required, a normalizing reference beam must be introduced. However, in our proof-of-principle setup, the direct absorption approach is sufficient.

Results and discussion

First, the chemical specificity is investigated with a sample consisting of pure olive oil. The sample is assembled without the spacer to avoid saturation of the absorbance. The wavelength dependent absorbance is measured by scanning the monochromator and taking the logarithmic ratio of the acquired spectrum and a spectrum taken without the sample. Figure 2 compares such an absorbance spectrum with a FTIR absorbance spectrum of a 4 μm thick oil sample. The shown absorption lines correspond to CH-stretch in the oil and a fine agreement is seen. In Fig. 2 is a FTIR absorbance spectrum of a 4 μm water sample shown for comparison.

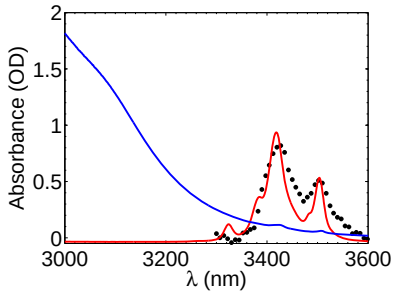

Fig. 2. Blue/Red line: FTIR absorbance spectrum for 4 μm thick water and oil samples respectively. Dots: Absorbance spectrum of oil made by scanning the grating and using the IR SC source.

The chemical specificity combined with spatial resolution is investigated with a sample consisting of an oil/water mixture. In Fig. 3a an optical microscope image of the area of interest is shown. Three areas are seen; a circular structure in the middle, one above and one below. It is impossible to distinguish these areas from each other with respect to what they consist of. The images in Figs. 3b and 3c are made by raster scanning the sample in steps of 5 μm at two wavelengths corresponding to high absorption in water and oil respectively. In this way two IR absorption images are obtained. Blue parts of the images indicate high absorption, and red parts low absorption. The absorption in oil is measured at 3.5 μm because the absorption in the water is smaller here than at the absorption maximum in oil around 3.42 μm. Figure 3b displays the water absorption. A high absorption is clearly seen in the upper part of the picture and points to the presence of water in this region. Figure 3c displays the oil absorption. Here we see high absorption in the lower part indicating that this is oil. The upper part shows some absorption and come from the tail of absorption in water at long wavelengths. The central part shows low absorption in both pictures and corresponds to an air bubble trapped at the interface.

Additional images have been made at 2.6 μm and 4.0 μm and these do not show any contrast. This underlines that the contrasts seen in the images are caused by absorption and that

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the contribution from refraction and scattering is minimal. It is clear that much more information about the sample is obtained from the IR images than from a normal optical image. As mentioned before, this chemical specificity is the main advantage of an IR microscope.

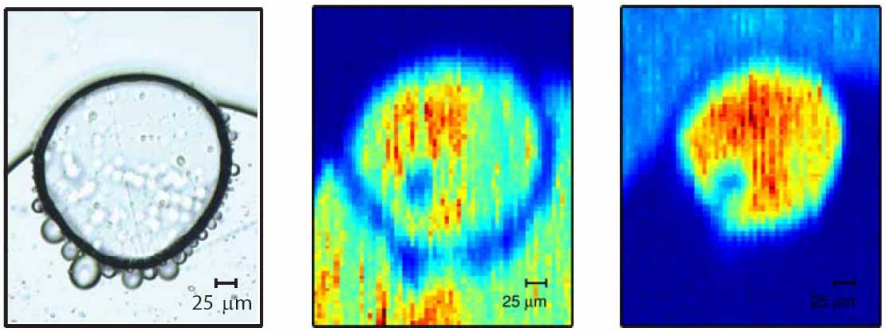

Fig. 3. (a) Picture of sample oil/water mixture made with an optical microscope. (b) and (c) IR images of sample measured at 3.05 μm and 3.50 μm corresponding to wavelengths of high absorption in water and oil, respectively. The blue color indicates high absorption and the red low absorption. The image size is $300\mu m \times 375\mu m$ and each pixel is $5\mu m \times 5\mu m$.

In an ordinary IR microscope the spatial resolution is determined by an aperture combined with an objective. In our demonstration setup there is no defining aperture and the spatial resolution is therefore completely determined by the spot size of the focused light. A theoretical investigation of the $1/e^2$ spot size is shown in the right part of Fig. 4. These calculated results are determined by propagating a Gaussian mode from the fiber output using ABCD matrices and the propagation law. As the starting beam profile, we use the wavelength dependent mode field from the ZBLAN fiber. This is found by using a commercially available finite element software package that includes the geometry of the fiber and the wavelength dependent refractive index of ZBLAN [21] (see insert in Fig. 4). Furthermore the chromatic aberration of the objectives is taken into account by using a wavelength dependent refractive index [22]. The calculation shows that the minimum beam radius as well as the focal distance are wavelength dependent. This is a manifestation of the chromatic aberration of the lenses and the wavelength dependent mode field radius of the fiber. The chromatic aberration could be circumvented by using reflective optics. Theory consequently shows that the fiber mode diameter is an important parameter for the spatial resolution and by choosing a fiber with a higher numerical aperture, one subsequently obtains a better spatial resolution in the IR microscope.

The left part of Fig. 4 shows a measurement of the spot size for three different wavelengths using the knife edge method. The data show the wavelength dependent variations in focal length and minimum spot size predicted by the model. The minimum measured beam radius is 16 μm and 12 μm at 2.8 μm and 3.8 μm, respectively. This is consistent with the calculation and even though the minimum beam radius does not coincide for the different wavelengths, the combined beam radius at the focal point still has a minimum about 17 μm. An estimate of the resolution can also be determined by considering the oil/air and water/air interfaces in Fig. 3 as knife edges. In this, way we obtain a resolution of 35 μm and 25 μm for the water absorption image and oil absorption image respectively. The resolution is slightly lower, than the limit imposed by the beam waist of the IR SC. This could indicate a small displacement of the sample with respect to the focal point of the microscope. The ability to focus the fiber based SC source to a small area while maintaining its full brightness and spectral bandwidth is crucial, as it provides a significantly higher intensity than provided by a thermal source. This enables faster raster

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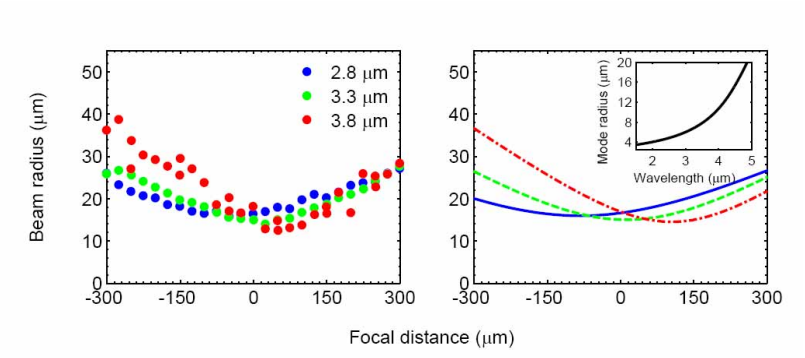

Fig. 4. The measured and calculated spot-sizes in the waist region of the IR microscope. The results are shown for three representative wavelengths. The inset shows the calculated mode field radius of the fundamental mode in the ZBLAN fiber.

scanning in high spatial resolution measurements.

Supercontinuum generation is noisy by nature [23] and the spectral density at a given wavelength will fluctuate somewhat. An investigation of the fluctuations is made by measuring the intensity at a single wavelength. A series of 500 points is recorded where each point is averaged over 15 ms. A signal to noise ratio (SNR) for the light source can be defined as the mean of this measurement divided by its standard deviation. A SNR of 100 is obtained at a wavelength of 3500 nm. This shows that the intensity fluctuations in the IR SC are low enough that it can be utilized in fast scanning instruments. Furthermore, by either normalizing or modulating the IR SC, SNR can be increased substantially. However, the fluctuations are not negligible and it is important to emphasize that the images shown are made without a normalizing reference beam. If a reference is introduced every single absorption pulse can be compared to the real spectrum allowing detection of much smaller absorptions.

Presently the wavelength range of the SC generated in ZBLAN is limited to the wavelength range covering primarily the stretching modes of O-H and C-H vibrations. However, fibers made of other soft glass materials such as tellurite or chalcogenide transmit light at longer wavelengths [24]. As an added quality, the nonlinearities of the tellurite and chalcogenide materials are orders of magnitude higher than that of ZBLAN. This improves the efficiency of the SC generation and relaxes the requirements for the pumping lasers. This shows promise of an infrared SC source extending into the molecular fingerprint region, which will give wide spread access to versatile high resolution infrared microscopy.

In conclusion, we have demonstrated that an infrared supercontinuum source based on an optically pumped ZBLAN fiber provides a simple, bright and broadband light source ideally suited for high resolution infrared microscopy. Even this proof of principle setup allows us to obtain images with a spatial resolution around 20 μm, and with chemical selectivity, based on the selective absorption of the infrared light by the different molecules in the sample. With the present pace in the development of high power fiber lasers and new materials for infrared optical fibers, the demonstration of IR microscopy based on supercontinuum sources will pave the way for a much more widespread application of IR microscopy as a supplement and complement to hyperspectral imaging in the visible and in coherent and incoherent Raman microscopy.

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