

# Cantilever Enhanced Photoacoustic Spectroscopy of Hair Microsamples

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## Abstract:

We have demonstrated that a highly detailed infrared spectrum of single human hair fiber with length less than 1 cm can be measured using cantilever based photoacoustic detection. The smallest measured hair sample was approximately 0.5 mm in length and 50  $\mu\text{m}$  in diameter. Different sample sizes have been measured and the effect of sample size to the signal-to-noise ratio (SNR) has been investigated. In addition, depth and length profiling spectra have been collected. Depth profiling experiments showed a change in the lipids of the hair in the different layers. Length profile of a longer hair fiber showed a change in the S=O peak at 1042  $\text{cm}^{-1}$ . A practical sample size for measuring single hair fibers with this setup was determined to be 5 mm. The method showed an excellent reproducibility of the measurement in addition to the high sensitivity of the cantilever microphone.

**Key words:** Photoacoustic spectroscopy, cantilever, hair, keratin, microsample.

## Introduction

Hair can be used as a matrix in various applications, for example in drug testing, toxicology and forensic sciences [1, 2]. Hair can be used in place of more commonly used blood or urine samples in many cases, and has some advantages like non-invasive measurements and larger detection window. In infrared spectroscopy, human hair has been normally studied by using diffuse reflectance infrared Fourier transform (DRIFT) or attenuated total reflectance (ATR), for which the sample preparation methods can lead to problems of reproducibility. Definite information could be obtained by studying intact individual hair fibres but small diameter of hair fibers and lack of sensitivity makes such measurement difficult. Photoacoustic spectroscopy (PAS) is a valuable tool for studying solid samples of various morphologies due to ease of sample preparation and depth profiling capabilities. However, the overall sensitivity of the photoacoustic method has not always been sufficient to make accurate measurements with human hair.

The pressure sensor, the condenser microphone that is commonly used in photoacoustic systems, mainly limits the sensitivity of the photoacoustic detection. The best condenser microphones work at the physical limits and therefore there is no way to

further improve their sensitivity. The interferometric cantilever microphone [3,4] can be constructed in such a manner that the sensitivity is even several orders of magnitude higher than the one of the condenser microphone. Due to its high sensitivity, PAS can work in a small scale formerly reached only by infrared microscopy. In the scale of photoacoustic spectroscopy, the samples are considered remarkably small and hence called microsamples.

In this study, microsampling capabilities of cantilever enhanced photoacoustic Fourier transform infrared (FTIR) spectroscopy are investigated with single human hair fibers by measuring different sample sizes and performing length and depth profiling experiments.

## Experimental

All samples were measured with a Bruker Tensor 37 FTIR and a Gasera PA301 photoacoustic detector. Parameters used in the measurements were 100 scans corresponding to 4 minutes measurement time per sample, 2.2 kHz scanner velocity and 8  $\text{cm}^{-1}$  resolution. Helium was used as a carrier gas in the cell for an enhanced signal and carbon black was used as a reference material. Samples were cut to size with scissors and put into sample holder with fine tweezers. No additional sample preparation was needed.

Tab. 1: Photoacoustic signal intensity of the Amide I peak and calculated SNR for a 5 mm hair sample when using different sample holders. Noise levels were determined by calculating peak-to-peak value over the region of 2200-2300  $\text{cm}^{-1}$ .

Sample holder type	Amide I peak intensity	SNR
Regular sample cup	0.42	23
Microsample accessory	1.19	107

Different sampling techniques of hair fibers were applied to achieve the best signal level in each measurement. An ordinary PA301 sample cup, a Tungsten needle and a fiber sampler of ST Japan microsampling kit, and a similar self-made fiber holder were used in the measurement of the samples. The ST Japan fiber holder is designed for 5 mm fibers while the self-made fiber holder has a sample size of 8 mm. Sample sizes smaller than 5 mm were measured with the Tungsten needle.

Different sampling techniques play a significant role when using microsamples, and are generally required to achieve a sufficient signal-to-noise ratio (SNR). When there is very little sample material, one has to be careful that most of the acoustic signal is transferred to the carrier gas. The RG-theory defines a boundary layer of surrounding gas working as a piston [5]. The thickness of this boundary layer is  $2\pi\mu$ , where  $\mu$  is the thermal diffusion length of the gas, given by eq. (1):

$$\mu = \alpha / \pi f^{1/2} \quad (1)$$

where  $\alpha$  is the thermal diffusivity of the carrier gas and  $f$  is the modulation frequency. For helium, and for 2.2 kHz optical velocity, the thickness of the boundary layer is approximately 3-4 mm in the fingerprint region of this study (1000-1700  $\text{cm}^{-1}$ ). This means that the sample has to be elevated a few millimeters from the surface of the sample cup in order to detect the maximum signal with no loss of heat to the sample cup. This criterion applies also for the cell window [6]. Moreover, the design reduces the cell volume for a higher signal. The benefit gained by elevating the sample can be seen in Tab. 1. For a 5 mm long single hair fiber, the signal intensity at Amide I peak (C=O stretch, 1660  $\text{cm}^{-1}$ ), which is the most pronounced feature in the spectrum, is almost three times higher and SNR over four times higher for the elevated sample. Achieving four times higher SNR generally means a 16-fold

measurement time. In the following experiments, special sample holders are used in all microsample measurements for a good thermal insulation.

## Results and Discussion

Sample size versus photoacoustic signal intensity was studied by measuring five different hair samples with different amount of sample material. Single hair fibers with lengths of 0.5, 2, 5, and 8 mm were measured and photoacoustic signal intensity was compared to the noise level of the used equipment. Moreover, a composition of hair fibers was measured as a comparison to the ordinary sampling techniques in solid phase PAS. A composition was prepared by adding 2-5 mm hair fibers to a PA301 sample cup until the sample level was almost flush with the edges of the cup. The measured spectra are displayed in Fig. 1. The features of the spectra are explained in a previous paper [7].

Fig. 1 shows that each measured sample size yields a detailed spectrum. The 5 and 8 mm samples can already be considered almost noiseless in the fingerprint region. With 0.5 and 2 mm samples, the intensity of the signal is moderate but the spectra are showing some level of interference induced by water vapor. The presence of water vapor could be avoided by thoroughly desiccating the photoacoustic cell and the optical beam path. From Tab. 2, it can be seen that relatively good SNR can be reached with all of the samples, and the detection limit of this setup obviously lies below the sample sizes used in this study. Different question however is, which sample size, SNR, and measurement time can be considered practical in each application. Nevertheless, a 5 mm single hair fiber should be enough sample material for this setup for a detailed analysis in most cases. When moving to a smaller sample size, the contribution of impurities and water vapor in the photoacoustic cell to the measured photoacoustic signal increases, which has to be taken into account, when analyzing the spectra.

Tab. 2: Amide I peak levels and calculated SNR for different microsamples. Noise levels were determined by calculating peak-to-peak value over the region of 2200-2300  $\text{cm}^{-1}$ . The peak-to-peak noise of 8 mm sample suffers from a varying baseline. A SNR of 140 is achieved for that sample, if the noise is calculated from a slightly different region.

	0.5 mm	2 mm	5 mm	8 mm
Amide I	0.59	0.90	1.24	1.67
SNR	38	76	107	88

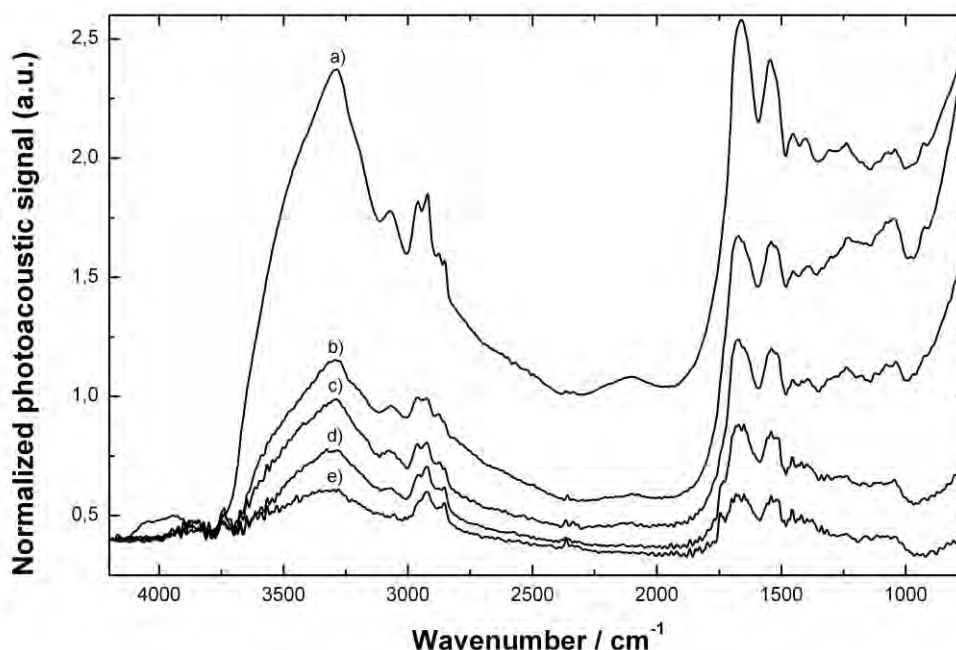


Fig. 1. Photoacoustic spectra of hair samples of different sizes. a) A composition of hair fibers, b) 8 mm, c) 5 mm, d) 2 mm, and e) 0.5 mm piece of fiber. The spectrum of a) was divided with a factor of 3 for a better comparison. The offset of the spectra was corrected at  $4200\text{ cm}^{-1}$ .

Achieving a sufficient reproducibility has been a problem in some of the earlier infrared studies of single fibers with different methods. Repeatability of this method with hair samples was investigated by measuring a same 5 mm sample 10 times. The sample was taken out from the cell and put back between every measurement. The variation in Amide I peak height was studied and a standard deviation as low as 0.98 % was achieved. In addition, the heterogeneity of different hair fibers from a same person was studied by measuring 10 different 5 mm hair samples. This time, the amide I peak variation was 4.59 %, which is still very much tolerable. The variation comes from slightly different composition between different hair fibers. A standard deviation less than one percent between different measurements and less than 5 % with different samples denote an excellent reproducibility. The situation can yet be improved by normalizing peak intensities.

Length profiling of a longer hair fiber was performed to study the weathering of the hair fibers by for example sunlight, rubbing, and washing. An 8 mm sample was taken at intervals of 2 cm from a longer hair fiber with an overall length of 40 cm. The first (root end) and last (tip end) 2 cm pieces were not measured. Thus, 18 different samples were acquired starting from the root and proceeding to the tip of the hair. The most significant feature of weathering in the infrared spectrum of hair is the increased intensity of the S=O peak at  $1042\text{ cm}^{-1}$  mainly due to photochemical degradation

of the amino acid cystine [7-9]. The alteration in the S=O peak throughout the length of the hair was quantified by setting a local minimum at  $950\text{ cm}^{-1}$  as zero level and comparing the height of the  $1042\text{ cm}^{-1}$  peak to the one of the nearest peak in  $1076\text{ cm}^{-1}$ , which remains almost unaltered regardless of which part the sample is collected. The calculated ratio for  $1042\text{ cm}^{-1}$  and  $1076\text{ cm}^{-1}$  peak intensities for all the samples can be seen in Fig. 2.

In Fig. 2, a clear rising trend can be seen, when moving towards the tip end of the hair. This indicates the increasing amount of S=O bonds in the older hair fiber material. The increased amount of S=O bonds appears due to oxidation induced by weathering. The ratio rises rapidly when moving from root end towards the tip end, but after approximately 10 cm of hair fiber, the ratio starts to saturate. 10 cm in length corresponds to a time span of roughly 240-290 days depending on the reference [8, 10]. The saturation of the ratio is a reasonable result, as there is only limited number of molecules to be oxidized. Weathering also happens mostly in the cuticle, which reduces the number of possible oxidized molecules.

Tab. 3: Thermal diffusion lengths in hair at  $3000\text{ cm}^{-1}$  with different optical velocities calculated from the constants used in [11].

Optical velocity	2.2 kHz	5 kHz	10 kHz
$\mu\text{ (}\mu\text{m)}$	4.0	2.6	1.9

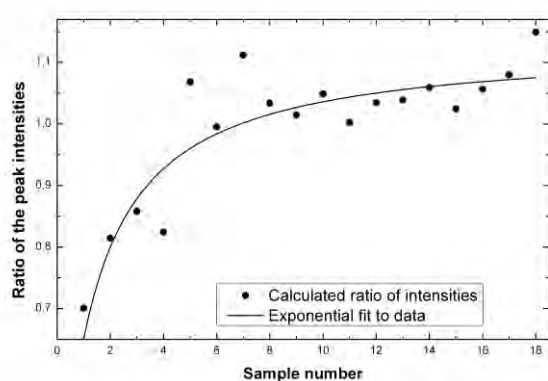


Fig. 2. The change in the ratio of the  $1042\text{ cm}^{-1}$  /  $1076\text{ cm}^{-1}$  peak intensities along a longer hair fiber.

Depth profiling capability of PAS is demonstrated with hair microsamples in Fig. 3. A same 8 mm sample was measured with 3 different optical velocities ranging from 2.2 to 10 kHz. With higher optical velocities, the number of scans was adjusted to maintain a constant measurement time. Thermal diffusion length is inversely proportional to the square root of the optical velocity, which means a smaller probing depth in the sample when increasing the scanning velocity of the FTIR mirror. Thermal diffusion lengths at  $3000\text{ cm}^{-1}$  for different optical velocities are calculated in Tab. 3. Depth profiling studies with hair fibers using PAS and ATR have also been previously done with different focuses [10-12].

In Fig. 3, a change in the peak intensities between  $2900\text{--}3000\text{ cm}^{-1}$  can be observed. The two peaks in this region belong to asymmetric stretching of  $\text{CH}_3$  in  $2962\text{ cm}^{-1}$  and  $\text{CH}_2$  in  $2918$

$\text{cm}^{-1}$ , which indicate the presence of lipids in hair [9, 10]. Two features can be discerned in the  $2900\text{--}3000\text{ cm}^{-1}$  region when moving to the higher scanning velocities: an overall increase of the signal level and a change in the ratio of the peak intensities. Increased signal level denotes more lipid content near the surface. This is most likely due to the stratum corneum lipid on the surface of hair [13]. These lipids give the unwashed hair its oily feel. Also the outermost layer of hair, the cuticle, contains 20-30 % lipids [8]. The altered ratio indicates the higher percentage of longer chain lengths of fatty acids near the surface of the hair. Similar features could also be observed in other  $\text{CH}_2$  and  $\text{CH}_3$  vibrations, involving symmetric stretches in  $2850\text{--}2875\text{ cm}^{-1}$  and symmetric and asymmetric bendings in  $1390\text{--}1450\text{ cm}^{-1}$ .

The fingerprint region  $1000\text{--}1700\text{ cm}^{-1}$  suffers of saturation effects, and therefore it is difficult to compare the spectra in this region. The situation could be improved by correcting the spectra using saturation compensation or linearization algorithms, but such algorithms are not examined within the framework of this study. The spectra could also be corrected locally using baseline correction and normalizing the peak heights if features of the fingerprint region were to be further investigated.

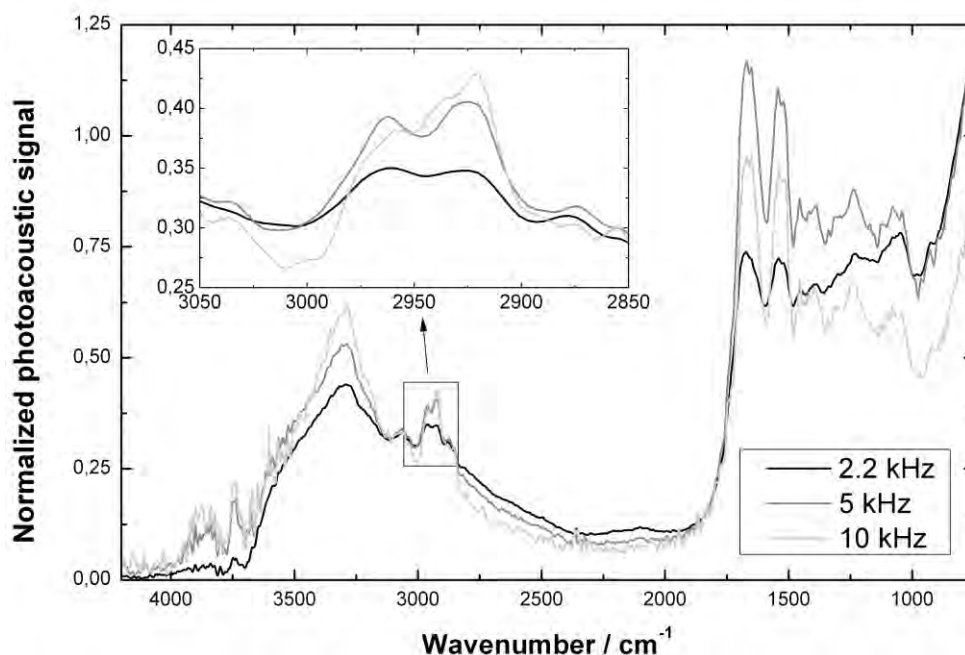


Fig. 3. Depth profiling performed on a single 8 mm piece of hair. Three different FTIR scanning velocities were used to achieve different probing lengths in the sample.

## Conclusions

Cantilever enhanced photoacoustic spectroscopy showed promising results with hair microsamples. Single hair fibers, as small as 0.5 mm in length, were successfully measured with a good SNR. Also, depth and length sensitive information could be obtained from single hair fibers. The method showed high sensitivity, but also an excellent repeatability of the measurements. The benefits of PAS in this application are the ease of sample preparation along with a depth sensitive, non-destructive, and non-invasive measurement. Photoacoustic measurement of hair microsamples could have applications in medical diagnosis, drug testing, and forensic and cosmetic sciences.

Different sources could also be applied in this study to improve the source power and hence the detection limit and SNR. For example, tunable external-cavity quantum-cascade lasers (EC-QCL) or optical parametric oscillator (OPO) lasers could be used in place of an FTIR device in specific applications. With a laser source, the overall instrument size could also be decreased to hand-held range in the future.

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