

# Quantum cascade laser based replacement for FTIR microscopy

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## ABSTRACT

Infrared (IR) microscopy has shown itself to be an important diagnostic tool for tissue analysis. To date, the main tool for performing IR microscopy has been the Fourier transform infrared (FTIR) microscope. FTIR microscopes utilize incandescent bulbs for light sources, and require cryogenically cooled detectors for the weak, optically poor probe signals. Image acquisition times can be tens of minutes even for sophisticated instruments, and the size and cost of FTIR microscopes precludes their broader clinical use. The development of broadly tunable, external cavity quantum cascade lasers (ECqCL<sup>TM</sup>) has created an ideal light source for IR microscopy. Spectrally brilliant probe beams that are diffraction limited, with intensities many orders of magnitude higher than incandescent sources, can be generated from compact, room temperature ECqCL<sup>TM</sup> devices. Moreover, the increase in intensity allows the use of room temperature microbolometer focal plane arrays (FPAs) for detection. The combination of ECqCLs<sup>TM</sup> and microbolometer FPAs opens the possibility of producing low cost, compact, room temperature IR microscopes with acquisition speeds thirty times that of state-of-the-art FTIR microscopes. The present study explores the challenges of creating this new generation of IR microscopes, and demonstrates the capabilities of the technology.

**Keywords:** microscopy, infrared, laser, quantum cascade, multispectral, imaging

## 1.

## INTRODUCTION

Biomedical imaging technologies are going through a period of rapid development that promises to make even the most sophisticated techniques available to not just first tier hospitals and research institutions, but clinics and doctors worldwide. This revolution has been fueled by advances in basic electronics, underlying science and technologies, computing power and algorithm development, and distribution of knowledge and information through the internet.[1-3] Examples of technologies that have become more accessible because of this revolution are optical tomography and ultrasonic imaging.[2] There is a class of biomedical imaging technologies, however, that has tremendous potential as a diagnostic aid, but has not yet seen the level of change necessary to bring them into the hands of the broader medical profession. Chemical imaging techniques are unique in their ability to provide not only spatial information, but chemical composition of the underlying substances being viewed.[4]

Infrared microscopy is a chemical imaging technique that is based on strong molecular absorptions in the mid-infrared (IR) spectral region (3 to 20  $\mu\text{m}$ ). These strong absorptions correspond to excitations of the fundamental vibrations in molecules. Since the frequency, strength, and distribution of these vibrations depends on the molecular structure and environment, mid-IR spectra can be used to uniquely identify different chemicals in both the gas and condensed phases. Infrared microscopes record the transmission or reflectance spectra at each spatial point in a sample.[5] This information can then be fed to a spectral analysis algorithm to develop a detailed map of chemical composition for an image, as shown in Fig. 1 from Diem *et. al.*[6, 7]

An area of medical diagnostics that is particularly suited to infrared microscopy is tissue analysis.[6] As shown in Fig. 1, different types of tissue can be uniquely identified in a way that is not possible with visual microscopy. This capability is extremely interesting for disease testing, in particular for cancer. Studies by Fernandez *et. al.*[8] show how infrared microscopy can be used to screen tissue samples for different conditions. At present, studies like this are performed in a research laboratory environment on equipment that may require 30 minutes or more to image a tissue sample. If these techniques could be streamlined, however, real-time screening of tissue samples could be implemented. This could have far-reaching impact in the field of medical diagnostics. Chemical imaging would increase the accuracy of the diagnostics, reducing false positives and the cost and anguish of further testing and treatment. Field doctors could more readily identify diseases like cancer, ensuring that treatment would begin earlier when it is more effective.

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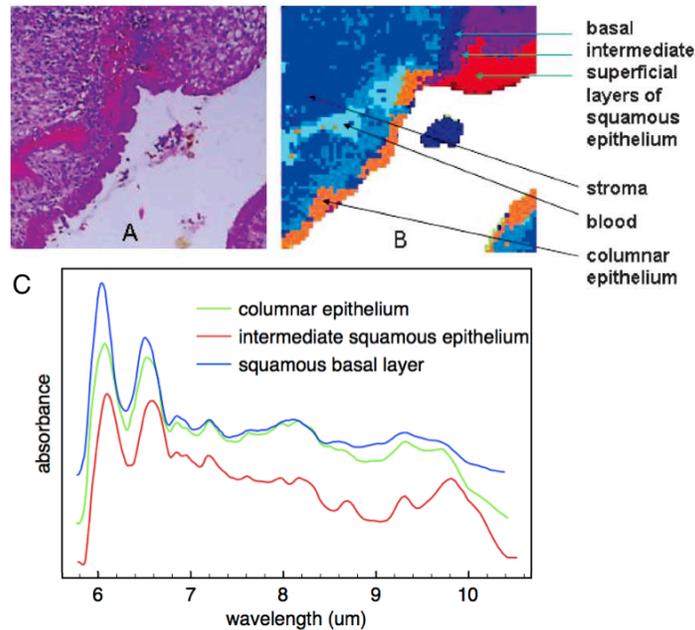


Figure 1. A) Photomicrograph of stained cervical tissue, B) same tissue chemically imaged using infrared microscopy, and C) examples of identifying infrared spectra. From Diem *et. al.*[6]

In spite of its potential, infrared microscopy has been largely confined to the research lab. A review of the technology shows why. The schematic for a standard Fourier transform infrared (FTIR) microscope is shown in Fig. 2. The traditional light source for performing mid-IR spectroscopy on samples has been the FTIR spectrometer. As shown, this consists of a broadband incandescent bulb with a poorly defined beam that must be conditioned before feeding it into a mechanical interferometer. A moving mirror creates the interferometric pattern in the probe light that can later be analyzed via Fourier transform to retrieve optical power as a function of wavelength. The light must then be further conditioned to couple into the microscope itself. The opto-mechanical assembly for generating the probe light alone can take up many liters of volume, and require numerous optics and careful free-space beam alignment. None of this is conducive to size and cost reduction, nor high volume manufacturing techniques.

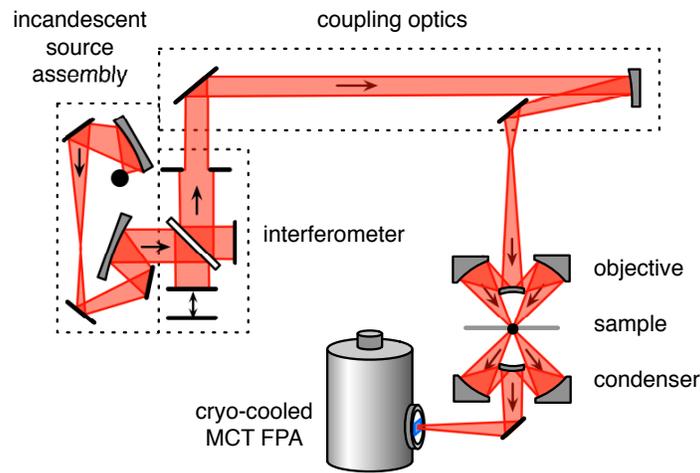


Figure 2. Schematic of a traditional FTIR infrared microscope configured for transmission measurements. Image acquired via a cryogenically-cooled focal-plane-array (FPA).[7]

The detection of the infrared probe beam in an FTIR microscope has been the other main limitation for this technology. The spectral brightness of incandescent sources is low, necessitating cryogenically cooled detectors for adequate signal to noise. Traditionally, liquid nitrogen cooled mercury-cadmium-telluride (MCT) detectors have been used.[5, 7, 9] Any

type of cryogenic cooling greatly reduces the commercial potential of an instrument; liquid nitrogen is not possible for portable or field clinic use, and cryo-coolers add cost, size, and power consumption to an instrument. Also, the cryogenically-cooled MCT array detectors necessary for rapid image acquisition[10] are prohibitively expensive and export-controlled by the U.S. State Department because of their military use. To date, the only alternative to incandescent lamps for FTIR systems has been synchrotron radiation,[7, 9] which is clearly not an option for commercial instruments.

The last twenty years have seen a revolution in mid-IR technologies that offer true potential for commercial instrumentation. On the source side, quantum cascade (QC) lasers have been developed. Starting with the potential of the first demonstration of QC lasing in 1994,[11] the technology has advanced to the point where QC gain media are capable of lasing at room temperature and supporting wavelength ranges that allow performing spectroscopy over a significant fraction of the mid-IR with only one laser.[12] Daylight Solutions has taken this technology and realized its commercial potential in a product line of broadly tunable external cavity QC lasers (ECqL™).[13] As shown in Fig. 3, battery powered laser systems built by Daylight Solutions are capable of tuning through the mid-IR spectral region necessary for tissue identification.

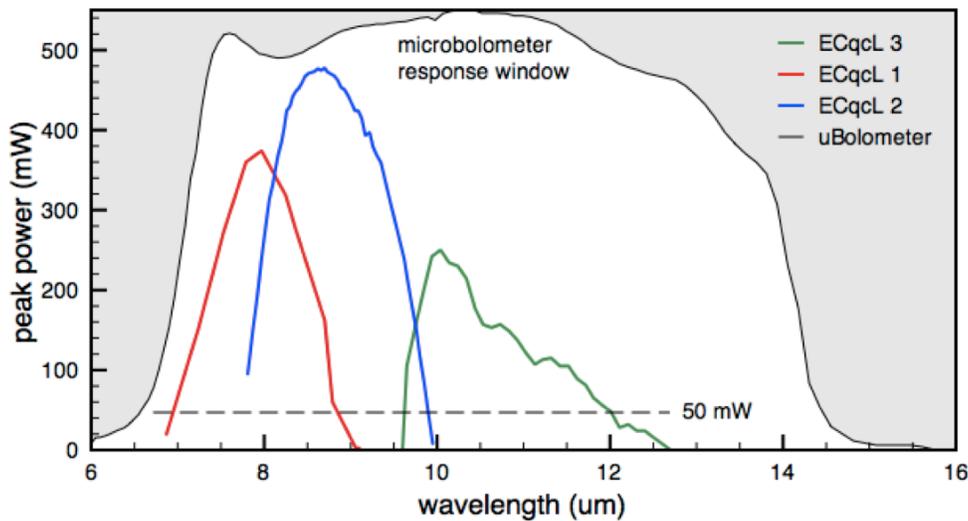


Figure 3. Demonstrated tuning ranges and peak power levels of Daylight Solutions external cavity QC lasers (ECqL™) compared to uncooled microbolometer FPA relative response (arb. units). Three lasers suffice to cover 7 to 12  $\mu\text{m}$  with at least 50 mW of peak pulsed power. Microbolometer response from J. Rice.[14]

Moreover, the high optical powers produced by QC lasers allow the use of new types of detectors, the other side of the equation. In particular, room temperature microbolometer focal plane arrays (FPAs) have been developed and commercialized[15] to the point where high resolution, battery powered modules can be purchased for under \$5,000. These focal plane arrays are not export controlled, and offer the potential for image acquisition speeds and resolution only obtainable with state-of-the-art cryogenically cooled MCT FPAs, provided that sufficient infrared probe signal is available.

As shown in Fig. 3, QC lasers and microbolometer FPAs are complimentary technologies that offer illumination and detection in a spectral range relevant to tissue identification. These technologies can be brought together to create a new infrared microscope that surpasses FTIR microscopes in performance, portability, manufacturability, and lower cost. There are many challenges to bring this about. Tunable QC lasers need to be developed that are optimized for imaging illumination and FPA hyperspectral data acquisition, and meet the requirements for low power consumption, manufacturability, and cost. Microbolometer FPAs need to be tested and modified to enhance performance for spectroscopy. Finally, a complete system has to be engineered that takes into account the unique requirements of a laser-based spectral imager.

## 2.

## TECHNICAL APPROACH

In order to create a replacement for the FTIR microscope, the elements of the microscope have to be understood in greater detail. In this section, technical details regarding infrared microscopes will be discussed. This will be followed by consideration of the candidate technologies to be used in a new infrared microscope, and the technical and commercial issues surrounding them. Finally the technical objectives necessary for a demonstration of feasibility will be presented.

### 2.1 Infrared Microscopes - Key Elements and Performance

As discussed above, the choices of infrared probe and detector have largely been driven by available FTIR spectrometer technology. This in turn has determined the ultimate performance available to FTIR microscopes. Consideration of the key performance requirements of spatial resolution and acquisition speed help highlight the issues and opportunities of infrared microscopy.

In the earliest FTIR microscopes, imaging arrays were simply not available. Instead, a large area point detector was used, and rastering techniques employed to create an image. Rastering requires using apertures to reduce the size of the illuminating and imaged light. Because the fundamental resolution available in a confocal microscope (as shown in Figs. 2 and 4) is approximately  $\lambda / 2$ , [9] ideally apertures 10  $\mu\text{m}$  or smaller are used. Incandescent lamps are extended sources, so the beam cannot be conditioned to both couple into the microscope and pass through such a small aperture without significant loss of power. Hence, trade-offs are often made to increase the light throughput by lowering the resolution, i.e., the aperture size is increased to 20  $\mu\text{m}$  or greater. Even with these trade-offs, it can still take 3 to 5 minutes to acquire the spectrum for a single point in an image. [5] The desire for higher resolution has driven the use of synchrotrons as the broadband infrared source instead of incandescent lamps. The beam quality of synchrotron radiation is much higher, allowing up to a thousand times more light to be passed through a 10  $\mu\text{m}$  aperture. [7, 9] This can reduce the acquisition time and increase the resolution, but a synchrotron is not a viable solution for commercial applications.

Mid-IR array detectors became available for microscopy work first in the 1990s. Cryogenically cooled MCT arrays have the best spectral coverage for FTIR methods, and linear arrays [16] and FPAs [10] have been coupled to infrared microscopes. The advantage of using a FPA is in acquisition speed; acquisition times are shortened by a factor of  $\sim 100$ . [7] The disadvantages of cryogenically cooled MCT FPAs are very high cost and export control by the U.S. State Department. Therefore, linear arrays of approximately 16 elements are the only practical solution for most commercial microscopes. Linear arrays still require some rastering, resulting in image acquisition times of 30 minutes or more. [8]

Interestingly, the advantages of synchrotron radiation disappear when going to array detectors in infrared microscopes. [9] Although the beam quality of synchrotron radiation is much higher than for incandescent sources, the total intensity is comparable when illuminating an entire sample. This means the overall illumination intensity is low for both incandescent and synchrotron sources, necessitating longer integration times even for the most sensitive FPAs; acquisition times are up to ten minutes per scan, [10] although work has been done to improve these speeds by up to 33%. [17] Also, present FPAs have pixel pitches of approximately 30  $\mu\text{m}$ , meaning that the ultimate  $\lambda / 2$  resolution is not available as it is with rastering techniques and synchrotron radiation, although the resolution for rastered incandescent sources is comparable.

The above discussion highlights the issues to consider for replacing the source and detectors in an infrared microscope. FPA detectors are desirable for their acquisition speed, but only if the cost and performance allow use in room temperature commercial instruments. The resolution of the FPA must be sufficient to match the performance of FTIR microscopes to be of use in tissue analysis. The light source must have much higher intensity than synchrotron and incandescent sources; higher intensity allows illumination of the entire sample at levels sufficient to allow use of less-sensitive room temperature FPAs. Finally, the light source must be compact, low cost, operate at room temperature, and have beam quality allowing coupling into the microscope with as few optical elements as possible. This reduces complexity, size, and cost for the final system.

### 2.2 Tunable QC Laser Light Source

As discussed above, QC lasers are the most commercially viable option for the light source in an infrared microscope. Certainly the performance of these devices meets the above criteria. Consider Fig. 4, where the power levels of incandescent “global” sources, synchrotron radiation, and QC lasers are compared. The data in Fig. 4 considers the spectral brightness, or power in a spectral bandwidth, since this is the most relevant power level for determining the signal to noise of the acquired spectrum. It also compares the amount of power that can be placed in a 10  $\mu\text{m}$  spot. As can be seen, the QC lasers offer an intensity that is many orders of magnitude higher than for the other two sources. The

intensity is also high enough that it exceeds the microbolometer noise floor by a significant margin, indicating that a QC laser source could be used in conjunction with a room temperature FPA.

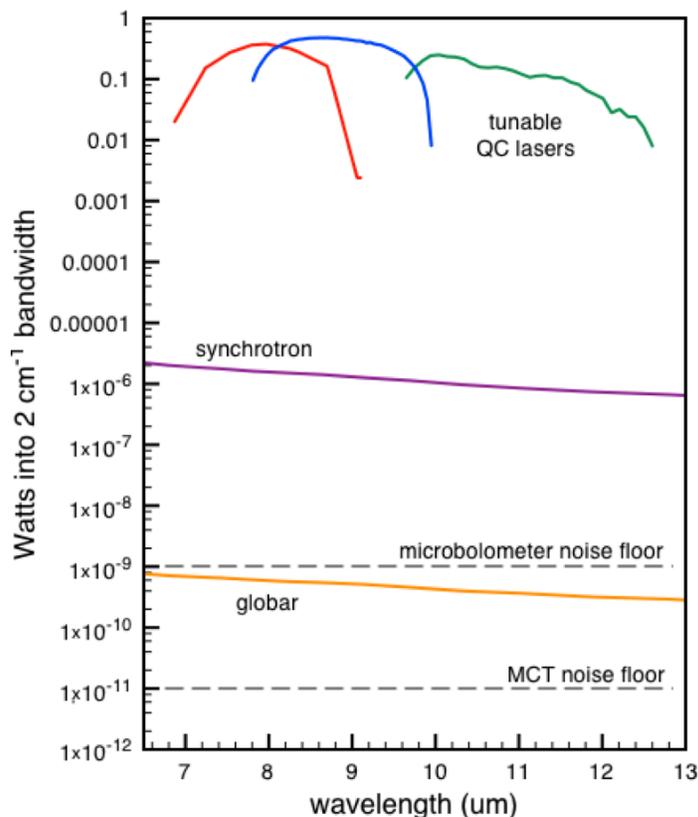


Figure 4. Comparison of signal level through 10  $\mu\text{m}$  pinhole for three different mid-IR sources. MCT noise level for liquid nitrogen cooled single element detector. Microbolometer noise level for uncooled FPA with 100 mK NEP. Globar and synchrotron data from Wetzel and Levine.[18]

The data in Fig. 4 needs some clarification with regards to FPA imaging. In the case of the synchrotron radiation and the QC laser, the beams are near diffraction limited, so it is possible to put almost all of the power into 10  $\mu\text{m}$ . The globar source, however, is not diffraction limited, so there is additional power outside of this area. If the illuminating beam is to be used for imaging with a FPA, the same intensity is to be spread out over the whole sample for the synchrotron radiation and QC laser, while the intensity for the globar across the sample will remain approximately equal to the 10  $\mu\text{m}$  value. Assuming a standard FPA array size, this means that the intensity will have to spread over approximately  $10^5$  pixels, with a reduction in intensity by a comparable amount. The synchrotron radiation intensity therefore only offers a signal-to-noise ratio (SNR) of approximately 10 with respect to the MCT noise floor. This helps explain the long integration times necessary for FPA image acquisition even with synchrotron radiation sources. For the QC laser however, a  $10^5$  reduction in intensity still leaves a SNR of  $10^3$  for a room temperature microbolometer FPA. This suggests that QC lasers are not only a viable replacement for the light source, but that SNR ratio is so much higher even with room temperature FPAs that acquisition times should be reduced drastically.

The potential for dramatically reduced acquisition times for QC lasers and FPAs meshes well with the way that data collection would proceed with tunable QC lasers. Assuming that an increase in SNR scales as the square of acquisition time, a 10 minute acquisition time with synchrotron radiation and a cryogenically cooled MCT FPA would be replaced by a sub-second acquisition time with a QC laser - literally a 10 Hz refresh rate. This in turn could be synchronized with both the FPA acquisition rate, and the laser tuning as it is scanned across the relevant mid-IR spectrum. Assuming a  $4\text{ cm}^{-1}$  resolution appropriate for condensed phase spectroscopy, a complete hyperspectral image could be acquired in just 20 seconds with the same level of sensitivity as demonstrated with present FTIR microscopes in 10 minutes, a thirty-fold increase in throughput potential for using infrared microscopes for tissue screening.

For all of the potential in using QC lasers for the light source, there remain two large challenges. The first has to do with using coherent laser light for illumination. Studies have shown that the mid-IR laser speckle that results from using coherent illumination can significantly reduce the resolution in imaging.[14, 19] Work needs to be done to understand the importance of laser speckle when QC lasers are used as the illumination source in an infrared microscope. Methods to remove the laser speckle potentially have to be developed to mitigate the possibility of reduced resolution. Work also has to be done to on how best to couple the tightly collimated radiation from the laser into the microscope. The optical coupling and laser speckle for imaging are related, and studies need to be performed with an actual FTIR microscope optical train and imaging apparatus to determine the issues that need to be addressed.

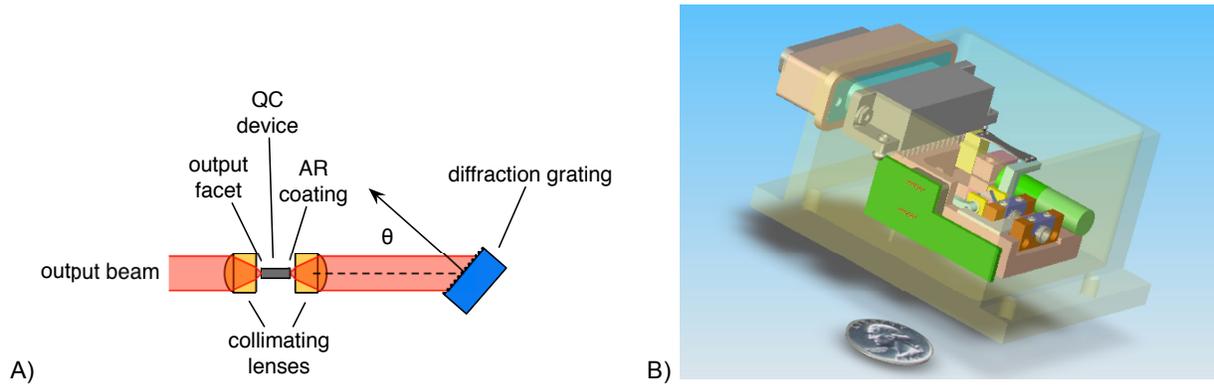


Figure 5. A) Schematic of external cavity QC laser with grating in Littrow configuration. Wavelength is tuned by changing grating angle. B) External cavity QC laser in final product form. U.S. quarter for size comparison.

The second main challenge is to develop an integrated QC laser system that meets size, power, and cost requirements appropriate for a commercial product, and has the necessary power and tuning characteristics to allow synchronization with a FPA detector for spectral acquisition. Daylight Solutions has developed ECqCL™ devices that can realize the full tuning range and power output of QC gain media. Fig. 5 contains a schematic of an external cavity, grating tuned laser, and the form it has taken when reduced to a commercial instrument. It needs to be determined how the ECqCL™ will be modified to meet the requirements for coupling to an infrared microscope and synchronizing with the data acquisition.

### 2.3 Room Temperature Mid-IR Detectors

As discussed above, room temperature microbolometer arrays have undergone a significant degree of commercialization compared to QC lasers. The practical factors of low cost, compact size, room temperature operation, and low power consumption make these imaging devices a natural fit for this application. The technical aspects of resolution and spectral response also mesh well with infrared microscope requirements. The main challenges with regards to FPA selection and integration will most likely be matching the detector dynamic range and laser power levels to achieve the most uniform illumination with the highest SNR, and developing algorithms to correct for changes in detector response over time and temperature shifts. Other issues to consider are extending the spectral response of these detectors, particularly to shorter wavelengths, and the practical aspects of synchronizing FPA acquisition with laser tuning.

## 3.

### SUMMARY

The key technology pieces of room temperature FPAs and compact tunable infrared lasers are ready to integrate to create an infrared microscope that has the potential for low cost and ease of use. Such a microscope could be of great service to medical clinics, offering cost effective and immediate tissue analysis in screening for cancer.

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