Cavity-Enhanced Absorption Spectroscopy of BChlα

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Abstract

Spectroscopy at the single-molecule level is a very useful technique for revealing molecular dynamics that are averaged out in bulk measurements. Absorption spectroscopy has several advantages over the more common fluorescence-based single-molecule studies: it eliminates shelving of fluorescent molecules and does not require fluorescent tags, making it a far less intrusive method. However, absorption measurements suffer from a high noise-background and rather low sensitivity. In our approach to absorption spectroscopy, a Fabry-Perot optical cavity greatly enhances the interaction between the photons and the molecules under study (in this case, bacteriochlorophyll-a, BChlα), resulting in higher sensitivity.

This paper describes the design of the cavity and the optical excitation and measurement apparatus, as well as first results of absorption measurements. The high-finesse cavity consists of closely spaced (∼100µm) mirrors, giving it wide free spectral range and fast response time. The cavity is scannable by piezoelectric actuators for mode-locking. It is contained in a chemically stable chamber that can be filled with the desired solution—in the case of these early measurements, with BChlα in acetone. For easy operation, the cavity is flushable with a pumping apparatus. The cavity mode volume is minimized for measurements on small numbers of molecules. In this project, preliminary absorption measurements were performed on as little as 10^6 BChlα molecules. It is estimated that this cavity can be used to make absorption measurements of less than ten molecules if other noise factors are reduced.

The longer-term goal is to observe conformational changes in BChlα while it is embedded in the cell membrane. In the more distant future, this method could be used to observe, in real time, many types of organic molecules as they undergo conformational changes in vitro. Combined with knowledge obtained through crystallography and direct mutagenesis, this information would greatly improve our understanding of many kinds of organic complexes and the processes in which they take part.
## Contents

Acknowledgements iii

Abstract iv

1 Introduction 1

1.1 Single-Molecule Detection: Historical Perspective . . . . . . . . . . . . . . . 1
1.2 Cavity-Enhanced Single-Molecule Absorption Spectroscopy . . . . . . . . . 2

2 Specific Goals 3

3 Theoretical Background 5

3.1 Resonance Cavity . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 5
3.1.1 Misalignment Effects in Stable Resonator . . . . . . . . . . . . . . . . . 10
3.2 Sensitivity . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 10
3.2.1 Feasibility of Experiment . . . . . . . . . . . . . . . . . . . . . . . . . 11
3.3 BChl\textsubscript{a} Absorption in Acetone . . . . . . . . . . . . . . . . . . 12

4 Experimental Apparatus 14

4.1 Optical Setup . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 15
4.1.1 Resonance Cavity Design . . . . . . . . . . . . . . . . . . . . . . . . . 17
4.1.2 Scanning the cavity . . . . . . . . . . . . . . . . . . . . . . . . . . . . 19
4.1.3 Interferometer . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 22
4.1.4 Sidebands . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 23
4.1.5 Detecting the Resonance Peak . . . . . . . . . . . . . . . . . . . . . . 23
4.1.6 Resonance Cavity Characterization . . . . . . . . . . . . . . . . . . . . 25
4.2 Cavity Alignment . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 30
4.3 Gas/Fluid Circuit . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 31
5 Results/Discussion 33

6 Conclusions 39

Bibliography 41
List of Figures

3.1 Fabry Perot Resonance Cavity. .......................................................... 5
3.2 Reflected intensity (without sidebands). The curve is narrower for \( F = 200 \) than for \( F = 100 \). .......................................................... 9
3.3 BChl\( \alpha \) absorption spectrum in acetone, obtained from the spectrophotometer. The scale on the vertical axis has an uncertainty of a factor of 3. .......... 13

4.1 The cavity sweeps through resonance. .................................................. 14
4.2 (a) Calculated resonance curve (using Mathematica), together with demodulated signal. (b) Sidebands allow scaling of the bandwidth. ................. 15
4.3 Optical setup. .................................................................................. 16
4.4 Sketch of cavity cell. (M1 and M2: cavity mirrors; C1 and C2: mirror mounts; K: Kalrez membrane; W: window; V1 and V2: valves; S: spring which pushes C2 against PZT). ......................................................... 18
4.5 High Voltage Amplifier. ................................................................. 20
4.6 Slip-stick friction between the mirror mount and the cavity casing is visualized with the interferometer setup. ................................. 22
4.7 Transmissivity (in \%) of mirrors. ......................................................... 24
4.8 The lower signal shows the sweep voltage to the piezo. As it decreases the cavity length, the resonance peak is overly narrow. As it expands the cavity, the peak is unusually broad. ......................................................... 25
4.9 The resonance peak is distorted as the cavity is expanded. ................. 26
4.10 The \( \Delta \nu_{FWHM} \) is larger if the cavity cannot reach equilibrium. .......... 29
4.11 Laser beam with wavelength outside the reflective region of the mirrors is incident on crooked cavity (exaggerated). Four reflections are created. To align the cavity, the input beam (3) must be aligned so that beams (1) and (5) are superimposed. 30

4.12 Setup for introducing BChl$\alpha$ and acetone solution and cleaning. 31

5.1 Plot of data collected. The plot shows measurements taken in air (A), in acetone (B), and in acetone + BChl$\alpha$ (C). 34

5.2 Measurements for (A) air and (B) acetone. Pairs of data show no significant difference in absorption. The data are in agreement with the average mirror reflectivity values advertised by the manufacturer of the mirrors, cf. Fig. 5.3. 34

5.3 Reflectivity (in %) of mirrors. 35

5.4 Plot of reduced data set. The plot shows measurements taken in air (A), in acetone (B), and in acetone + BChl$\alpha$ (C). 36

5.5 Measurements for (A) air and (B) acetone. Pairs of data show no significant difference in absorption. The solid line marks the reflectivity curve of an average mirror, plotted also in Fig. 5.3. 36

5.6 Extinction coefficients obtained for BChl$\alpha$. 36

5.7 Extinction coefficients for BChl$\alpha$ compared to extinction coefficients measured in spectrophotometer experiment given by the solid line (see Fig. 3.3). 37

5.8 $\Delta\nu_{FWHM}$ measured within trials have low errors. 38
List of Tables

5.1 Extinction coefficients obtained for BChla ................................. 37
Chapter 1

Introduction

1.1 Single-Molecule Detection: Historical Perspective

Most spectroscopic measurements of condensed matter measure only the average behavior of a large ensemble of molecules. At the same time, most theoretical models are limited to describing the behavior of single molecules. This gap between theory and experiment has narrowed considerably over the past decades through advances in single molecule spectroscopy (SMS). The field came into existence with the pioneering work by Hirschfeld, who in 1976 demonstrated the detection of a single antibody molecule stained with 80 to 100 fluorophores [4]. Progress in the field accelerated notably in 1989 when Moerner and Kador [7] proved a novel approach to condensed phase spectroscopy. The team used two double-modulation absorption methods to observe the optical-absorption spectrum of single dopant molecules in a crystal. One year later, Orrit and Bernard reported a fluorescence experiment on the same system that achieved SMS with far greater sensitivity. Also, that same year, the first single-molecule detection in liquid was achieved by Keller and collaborators [10]. Recent advances in microscopy and optics now allow laboratories around the world to perform SMS in many different systems.

SMS has two distinct advantages over ensemble spectroscopy. Firstly, it can resolve *temporal heterogeneity*. Single molecules can exist in many possible conformational states inside a particular solvent environment. In bulk measurements, these states are averaged so that dynamic or mechanical properties of the molecule are obscured. SMS can resolve these states, revealing interesting transitions and electron transfer mechanisms. Secondly, SMS resolves *spatial heterogeneity*, i.e., inhomogeneity of the local environment. This information is again lost in ensemble spectroscopy where one molecule is masked by many others in its
SMS today relies almost exclusively on fluorescence. In many cases, this technique presents disadvantages. In fluorescence, a molecule is excited to a higher energy state through a photon absorption. The excited state $A^*$ relaxes almost instantaneously with the emission of a photon: $A^* \rightarrow A + h\nu$. In many cases, the molecule can ascend to a higher energy state from which relaxation occurs many orders of magnitude more slowly via phosphorescence or non-radiative pathways. This phenomenon, known as “shelving,” can render a large proportion of the population invisible in the fluorescence measurement. Often researchers must resort to tagging the molecule with a fluorescent substance to make shelving less likely\(^1\). In many cases, the molecule under study even needs to be chemically altered to increase its fluorescent yield. SMS experiments that require such alterations of the molecule population clearly lose some of their credibility.

1.2 Cavity-Enhanced Single-Molecule Absorption Spectroscopy

In view of these problems, a non-intrusive technique for SMS studies would be extremely useful. Absorption spectroscopy could be the answer. Unfortunately, absorption measurements still suffer from a high noise-background and rather low sensitivity, making them impractical for spectroscopy at or near the single-molecule level. This project aims to improve these sensitivity issues. By placing the molecule population in a Fabry-Perot resonance cavity, the interaction time between the molecules and the photon bath be greatly enhanced while reducing the noise floor, so that the traditional hurdles of absorption spectroscopy on small ensembles can be overcome and single-molecule absorption spectroscopy (SMAS) achieved.

---

\(^1\)The most common tag is the green fluorescent protein with a quantum yield near 10% [13]
Chapter 2

Specific Goals

As the first step towards realizing SMAS, this project’s aim is to build a the resonance cavity and prove that increased sensitivity in absorption spectroscopy can be achieved. We chose to study bacteriochlorophyll-a (BChl\textsubscript{a}). This molecule is well-documented but still has some outstanding questions regarding its conformational response to photon absorption, which is particularly interesting in its role as light absorbers in plants. Even though the initial aim is for bulk measurements with enhanced sensitivity, the cavity is designed to be suitable for absorption measurements near the single-molecule level.

The primary aim of the experiment is to find the absorption extinction coefficient of BChl\textsubscript{a} from as few molecules as possible. First, we need to find the absorption coefficients for (i) air, (ii) acetone, and (iii) BChl\textsubscript{a} in acetone. These are calculated from (see Sec. 3.1):

$$\delta = 2\pi \frac{\Delta \nu_{FWHM}}{\Delta \nu_{FSR}},$$ \hspace{1cm} (2.1)

where $\Delta \nu_{FWHM}$ and $\Delta \nu_{FSR}$ are determined experimentally. The absorption coefficient for BChl\textsubscript{a} is then determined from

$$\delta_{BChl a} = \delta_{BChl a + acetone+mirrors} - \delta_{acetone+mirrors}$$ \hspace{1cm} (2.2)

Finally, the molar extinction coefficient $\varepsilon$ for BChl\textsubscript{a} is found from the Beer-Lambert Law, [2]

$$\varepsilon = \frac{\delta_{BChl a}}{2\ln 10 L C_{BChl a}},$$ \hspace{1cm} (2.3)

where $C_{BChl a}$ is the molar concentration of BChl\textsubscript{a} in acetone. Using a commercial spectrometer, a value of $\varepsilon \approx 36 \text{mM}^{-1} \text{cm}^{-1}$ was found, though the actual value is probably
higher (see Sec. 3.3). In Sec. 3.2, we calculate the sensitivity that can be achieved with the resonance cavity made in this project. We estimate that it is easy to observe 300,000 molecules, though it should be possible to observe as few as 30,000 with the current design.
Chapter 3

Theoretical Background

3.1 Resonance Cavity

The simplest resonance cavity consists of two curved, facing mirrors, separated by a distance $L$, shown in Fig. 3.1. The mirrors are characterized by their radius of curvature $\rho_c$, their diameter $d_{\text{mirror}}$, as well as their reflectivity $R_m = I_{\text{reflected}}/I_{\text{incident}}$, transmissivity $T_m = I_{\text{transmitted}}/I_{\text{incident}}$, and loss $L_m = 1 - R_m - T_m$. In addition, it will be convenient to consider the “mirror coupling coefficient” $\delta = 1 - R_m$.

The cavity can trap light if the curvature of the mirror and the distance between them is chosen appropriately. Many different resonance modes can be excited inside the cavity. We will limit the discussion to the lowest-order gaussian mode whose profile has no nodes. As the mode with the most highly confined transverse field and the lowest loss (\cite{11}, p. 412) it will be the resonance that we will use for the absorption measurements. The shape of this mode can be derived from the Helmholtz equation $\nabla^2 \psi + K^2 \psi = 0$, subject to the boundary given by the cavity geometry. This equation has a solution that is a gaussian

![Figure 3.1: Fabry Perot Resonance Cavity.](image-url)
beam ([6], p. 176):

\[
\psi_g = \frac{e^{Kz_0}}{z - iz_0} e^{iK[z + z_0\rho^2/2(z^2 + z_0^2)]} e^{-Kz_0\rho^2/2(z^2 + z_0^2)}.
\tag{3.1}
\]

Here, \( z \) is the distance along the cavity axis, \( \rho \) is the transverse distance, and \( z_0 \) is given by the radius of curvature of the gaussian beam:

\[
R(z) = z + \frac{z_0^2}{z},
\tag{3.2}
\]

The mode resonates inside the cavity if \( R(z) \) is matched to the mirror radii of curvature:

\[
R(z_1) = \rho c_1 = z_1 + \frac{z_0^2}{z_1}
\tag{3.3}
\]

\[
R(z_2) = \rho c_2 = z_2 + \frac{z_0^2}{z_2}
\tag{3.4}
\]

where \( z_1 \) and \( z_2 \) are the mirror positions.

To get an idea of how thick this resonant beam is, one considers the transverse distance at which the amplitude of the gaussian beam in Eq. 3.1 has decreased by a factor of \( \frac{1}{e} \). This distance is called the beam’s waist and is given by

\[
w = w_0 \sqrt{1 + \frac{z^2}{z_0^2}}
\tag{3.5}
\]

\[
w_0 \equiv \frac{\sqrt{2z_0}}{K},
\tag{3.6}
\]

where \( w_0 \) is the minimum waist at the center of the cavity. With \( K = \frac{2\pi}{\lambda} \) at resonance, \( w_0 \) can be written as

\[
w_0^2 = \frac{\lambda}{2\pi} \sqrt{L(2\rho - L)}
\tag{3.7}
\]

We will need to know how long light is resonant within the cavity and what fraction of the incident light is transmitted or reflected, and with what phase. To answer these questions, we can work with plane waves instead of the lowest-order Gaussian waves. The outcomes are the same for both since they are equally well reflected and transmitted.
So assume the electric field of the incident beam is

$$E_{\text{inc}}(t) = E_0 e^{i\omega t}$$

(3.8)

The electric field is transmitted and reflected with parameters $r = \sqrt{R_m}$ and $t = \sqrt{T_m}$ (since the electric field is proportional to the square root of the intensity.) From Fig. 3.1, it is easy to see that the electric fields of the reflected and transmitted components are given by

$$E_{\text{tr}}(t) = E_0(t_1t_2e^{i\omega(t-L/c)} + t_1t_2r_1r_2e^{i\omega(t-3L/c)} + t_1t_2r_1^2t_2^2e^{i\omega(t-3L/c)} + \cdots)$$

$$= E_0t_1t_2e^{i\omega(t-L/c)} \sum_{n=0}^{\infty} (r_1r_2)^n (e^{-2i\omega L/c})^n$$

$$= \frac{E_0t_1t_2e^{i\omega(t-L/c)}}{1 - r_1r_2e^{-2i\omega L/c}}$$

$$E_{\text{ref}}(t) = E_0(r_1e^{i\omega t} + (-r_2)t_1^2e^{i\omega(t-L/c)} + (-r_1)(-r_2)^2t_1^2e^{i\omega(t-2L/c)} + \cdots)$$

$$= E_0e^{i\omega t}(r_1 + t_1^2(-r_2)e^{-2i\omega L/c} \sum_{n=0}^{\infty} (r_1r_2)^n (e^{-2i\omega L/c})^n)$$

$$= E_0e^{i\omega t}(r_1 - t_1^2r_2 \frac{e^{-2i\omega L/c}}{1 - r_1r_2e^{-2i\omega L/c}})$$

Thus,

$$\frac{I_{\text{tr}}}{I_{\text{inc}}} = \left| \frac{E_{\text{tr}}}{E_{\text{inc}}} \right|^2 = \frac{t_1^2t_2^2}{1 + r_1^2r_2^2 - 2r_1r_2\cos(2\omega L/c)}$$

(3.9)

(3.10)

For the reflected term, we will be able to assume that the mirrors have equal reflectivity $r$. We will furthermore assume zero loss (which makes the calculations considerably easier, but doesn’t change what’s happening qualitatively.) The reflection ratio is then given by [1]

$$F = \frac{r(e^{i\phi} - 1)}{1 - r^2e^{i\phi}}$$

(3.11)

so that the intensity ratio is
\[
\frac{I_{tr}}{I_{inc}} = |F|^2 = r^2 \frac{2 - 2 \cos \phi}{1 + r^4 - 2r^2 \cos \phi}
\]
(3.12)

where \( \phi = 2\pi \frac{f}{\Delta \nu_{FSR}} = 2\pi \frac{2L}{\lambda} \) is the phase gained from one round-trip in the cavity.

The cosine terms in the denominators of Eqs. 3.12 and 3.9 show that these equations do in fact represent resonance curves. The peak of these curves occurs where the denominator is minimized, i.e., when

\[
2\omega L/c = 2\pi k, k \in \mathcal{N},
\]
(3.13)

or

\[
k\lambda_k = 2L, k \in \mathcal{N}.
\]
(3.14)

We will be most interested in the reflection. This is plotted in Fig. 3.2. The phase of the reflection curve, \( \xi = \arctan \frac{\Im [E_{ref}(t)]}{\Re [E_{ref}(t)]} \), is shown in Fig. 3.2 (b). One can think of the reflected term as made up of two contributions. One is the directly reflected beam, the other the leakage term, i.e., the fraction of the resonant beam that is transmitted back through the first mirror. If the cavity is well aligned and mode-matched, the intensity of the resonant beam is many times larger than the incident beam, so that the reflected beam has an intensity similar to that of the incident beam. On resonance, the reflected beam is \(180^\circ\) out of phase with the leakage term out of the cavity. The two reflected electric field components then interfere destructively so that the reflected beam intensity drops to a minimum. If the reflection drops to zero, the cavity is said to be impedance matched (as in a transmission line).

To investigate the behavior near resonance, it is convenient to expand the cosine term in Eqs. 3.12 and 3.9 around \( \omega_k = 2\pi \frac{c}{\lambda} \):

\[
2r_1r_2 \cos(2(\omega + \Delta \omega)L/c) = 2r_1r_2(1 - \frac{1}{2} \left( \frac{2L}{c} \Delta \omega \right)^2 + \cdots)
= 2r_1r_2(1 - \frac{1}{2} \left( \frac{\Delta \omega}{\Delta \nu_{FSR}} \right)^2 + \cdots),
\]
(3.15)

where we define

\[
\Delta \nu_{FSR} = \frac{c}{2L}
\]
(3.16)
Thus, Eqs. 3.9 and 3.12 become

\[
\frac{I_{tr}}{I_{inc}} = \frac{t_1^2 t_2^2}{(1 - r_1 r_2)^2 + r_1^2 r_2^2 (\Delta \omega / \Delta \nu_{FSR})^2} \tag{3.17}
\]

\[
\frac{I_{ref}}{I_{inc}} = r_2 \frac{(\Delta \omega / \Delta \nu_{FSR})^2}{r_4^2 - 1 + r^2 (\Delta \omega / \Delta \nu_{FSR})^2} \tag{3.18}
\]

The FWHM width of the resonance curve is

\[
\Delta \nu_{FWHM} = \frac{c}{2 \pi p} \sqrt{\frac{2 t_1^2 t_2^2 - (1 - r_1 r_2)^2}{r_1^2 r_2^2}} \tag{3.19}
\]

As a measure of the quality of the optical resonator, it is convenient to consider the finesse \(^1\)

\[
\mathcal{F} = \frac{c}{2p \Delta \nu_{FWHM}} \tag{3.20}
\]

\[
= \frac{\Delta \nu_{FSR}}{\Delta \nu_{FWHM}} \tag{3.21}
\]

It will be useful to relate the finesse directly to the total round-trip loss factor \(\delta_c\) ([11], p. 55):

\[
\mathcal{F} \approx \frac{2 \pi}{\delta_c} \tag{3.22}
\]

\(^1\)For a passive Fabry Perot Cavity, the finesse can be expressed in terms of the more common quality factor \(Q\) ([11], p. 430):

\[
Q_c = \frac{2L \mathcal{F}}{\lambda}
\]
3.1.1 Misalignment Effects in Stable Resonator

Misalignment or misadjustment of the mirrors can tilt and translate the optical axis of the resonator. Let $\theta_1, \theta_2$ and $x_1, x_2$ be the angular and translational misalignments of the two mirrors, respectively. If the axis shifts are large enough so that the beam center at the mirrors comes within a few spot sizes $w$ of the rim of the mirror, then aperture losses to the beam intensity can be significant. Therefore, we must ensure that

$$\frac{d_{\text{mirror}}/2 - \Delta x_i}{w} >> 1.$$  \hspace{1cm} (3.23)

From ([11], p. 768),

$$\Delta x_1 = -\frac{\rho_m}{2 - L/\rho_m}(\theta_1(1 - L/\rho_m) + \theta_2)$$ \hspace{1cm} (3.24)

$$\Delta x_2 = -\frac{\rho_m}{2 - L/\rho_m}(\theta_1 + (1 - L/\rho_m)\theta_2)$$ \hspace{1cm} (3.25)

For the cavity used in this experiment, $L \approx 500 \mu \text{m}, \rho_m = 5 \text{ cm}$, so we can approximate

$$\Delta x_1 = \frac{\rho_m}{2}(\theta_1 + \theta_2)$$

$$\Delta x_2 = \frac{\rho_m}{2}(\theta_1 + \theta_2)$$

Now, even if $\theta_1 \approx \theta_2 \approx 10^\circ$, condition 3.23 will be satisfied.

3.2 Sensitivity

We can approximate the number $N$ of $BChl a$ molecules that is required for absorption spectroscopy measurements. Assume a fractional detection sensitivity of $\eta = \frac{\delta_{BChl a}}{\delta_{acetone+mirrors}}$.

Then

$$\delta_{BChl a} = 2 \ln 10 L \varepsilon C$$

$$= 2 \ln 10 L \varepsilon \left(\frac{N/N_A}{L \pi w_0^2}\right),$$

or

$$\frac{N}{N_A} = \frac{\delta_{BChl a} \lambda \sqrt{L(2\rho - L)}}{4 \ln 10 \varepsilon}$$ \hspace{1cm} (3.26)
Note that \( L \geq 2\sigma \), where \( \sigma \) is the depth of one of the mirrors, \( \sigma = \frac{r_m^2}{2F} \). Assume a very conservative value of \( \eta = 0.5 \) (see Sec. 5). The parameters for the cavity are \( \rho = 5 \text{ cm}, \quad F \approx 100,000, \quad \delta_{\text{air}} \approx \delta_{\text{acetone}} \approx \frac{2}{F} \approx 10^{-5} \), and \( L \approx 450 \mu\text{m} \) (Sec. 4.1.1). These values show that we can easily take absorption spectroscopy on \( N \approx 300,000 \) BChl \( \alpha \) molecules. It is estimated that if the current design is supplemented with a high-frequency amplifier before the oscilloscope, the sensitivity can be brought down to \( \eta \leq 0.01 \), which puts \( N \leq 30,000 \).

### 3.2.1 Feasibility of Experiment

As mentioned earlier, this cavity is part of a longer-term experiment that will try to detect single molecules inside the beam. We will do a quick calculation to see if this cavity can in fact be used for this function. The fundamental problem is whether a single BChl \( \alpha \) will absorb enough light to make a detectable difference in the cavity output beam intensity, \( I_{\text{trans}} \). We will estimate what precision is required in the measurement of \( I_{\text{trans}} \). Suppose there are \( N \) BChl \( \alpha \) molecules in the beam. Then we require a fractional precision better than

\[
\eta = \frac{I_{\text{trans}}(N = 0) - I_{\text{trans}}(N = 1)}{I_{\text{trans}}(N = 1)}
\]

From [11], \( \frac{I_{\text{trans}}}{I_{\text{incident}}} \approx \frac{1}{(1+R)^2} \), where \( R \) is the ratio of the fractional absorption per cycle of one BChl \( \alpha \) molecule to that of the mirrors, \( R = \frac{\delta_{\text{BChl}}}{2\delta_{\text{mirror}}} \). From this, we obtain \( \eta = 2R + R^2 \approx 2R \) since \( R \ll 1 \), and thus \( \eta \approx \frac{\delta_{\text{BChl}}}{\delta_{\text{mirror}}} \). Note that we neglected the losses in acetone in this calculation; acetone does not absorb in the frequency range we use.

From the Beer-Lambert Law, we have \( \delta_{\text{BChl}} = 2 \ln 10 \epsilon C L \), where \( C \) is the molar concentration \( C = \frac{(1/N_A)}{V} \) with \( V \) the beam volume. If the cavity spacing is adjusted to 20\( \mu \text{m} \) (which is possible with the current design in conjunction with the soft PZT (see Sec. 4.1.2)), using mirrors with 5cm radius of curvature, a mirror finesse \( F = 100,000 \) (which is also possible if the mirrors in the current design are replaced with new ones) as well as \( \epsilon = 50 \text{mM}^{-1} \cdot \text{cm}^{-1} \) at 770 nm, these calculations yield \( \eta \approx 4 \cdot 10^{-6} \).

Attaining such sensitivities is not easy, but possible if the output signal is detected near the shot-noise limit. It can be done using hetero- or homodyning methods. Of course, other noise sources need to be minimized first. We have done this as much as it is possible at

\footnote{The mirror has the shape of a parabola given by \( y = \frac{1}{4f}r^2 \) where \( f \) is the focal length. Some geometry shows that \( \rho = 2f \), so that \( y(r = r_m) \equiv \sigma = \frac{\rho^2}{2F} \).}
this stage, using optical intensity stabilizers as well as an optical isolator, and mounting the apparatus on the same stable optical table. Since no transmitted light signal is available, the reflected signal must be used for measurements and alignment. Major noise sources include vibrations of the cavity, back-reflection through the setup, as well as noise in the laser input signal from the Ti-Saph laser.

3.3 BChl\textsubscript{a} Absorption in Acetone

There are many photo-active molecules that we could have studied. We chose BChl\textsubscript{a} for three primary reasons. Firstly, BChl\textsubscript{a} is a molecule that has long been studied in biology, so that there exists a great wealth of information on its properties. Secondly, one of the absorption peaks lies at $\lambda \approx 770$ nm. This is a wavelength we can produce with an already existing tunable single mode laser. Thirdly, and most importantly, BChl\textsubscript{a} is an interesting molecule from a biological viewpoint. Even though it’s been studied extensively, it is still not understood exactly how it functions in photosynthesis. Bacterial reaction centers contain two quinone ($Q$) cofactors, $Q_A$ and $Q_B$. When light strikes the reaction center, an electron is transferred from the BChl\textsubscript{a} dimer ($P$) to the two quinones in sequence:

$$PQ_AQ_B + h\nu \rightleftharpoons P^+Q_A^-Q_B \rightleftharpoons P^+Q_AQ_B^-$$  \hspace{1cm} (3.27)

Several relevant time scales in this process are still not known at this point. Recent research by Tiede and colleagues has suggested that there are actually two distinct populations of the bacterial reaction centers that differ in their $Q_A^-Q_B \rightleftharpoons Q_AQ_B^-$ transfer rates[12]. These populations are different configurational states of the same molecule, but the individual states are concealed through thermal averaging. One of the long-term goals of this project is to time-resolve single reaction centers to observe the dynamics between these different states.

In the current project, we attempt to reproduce the shape of the absorption peak at $\lambda = 771$ nm, depicted in Fig. 3.3. Absorption at this wavelength promotes an electron transition to the $\pi^*$ excited state.

Acetone is chosen as a solvent because BChl\textsubscript{a} dissolves in it and absorbs minimally near $\lambda = 770$. The spectrum of acetone was taken with a commercial spectrophotometer
Figure 3.3: BChl\textsubscript{a} absorption spectrum in acetone, obtained from the spectrophotometer. The scale on the vertical axis has an uncertainty of a factor of 3.

Instrument\textsuperscript{3}. The extinction coefficients obtained this way are shown in Fig. 3.3. It must be noted that the scaling in this figure is most likely inaccurate. Impurities in the sample of BChl\textsubscript{a} powder used in this measurement most likely caused the actual concentration of BChl\textsubscript{a} to be less than assumed for the calculation (Eq. 2.3) to scale this plot. Thus, the actual extinction coefficient of BChl\textsubscript{a} is probably somewhat larger than given here.

\textsuperscript{3}Agilent Technology, Inc.: UV VIS Spectrophotometer.
Chapter 4

Experimental Apparatus

We need to measure both the free spectral range $\Delta \nu_{FSR}$ and the bandwidth $\Delta \nu_{FWHM}$. The free spectral range is obtained in one of two ways. Either, the cavity length is moved through one free spectral range by moving one of the mirrors a distance $\lambda/2$, or the laser frequency is changed by $\Delta \nu_{FSR}$.

The bandwidth is measured from the resonance curve, which also can be obtained in two ways. Either, the frequency of the laser is swept over the resonance frequency while the cavity reflection is monitored. Alternatively, the cavity length is swept across the resonance length (i.e., across $L = k\lambda/2, k \in \mathbb{N}$). With either method, a Lorenzian resonance curve is obtained, as depicted in Fig. 4.1. The resonance curve is inverted since we’re looking at the reflection (see Sec. 3.1).

The difficult part is scaling the time axis in Fig. 4.1 so that $\Delta \nu_{FWHM}$ can be expressed as a frequency. A convenient way to scale is to introduce sidebands of frequency $\Delta \nu$ to the laser beam to the laser frequency $\nu$. If one is now sweeping the input frequency, one of the sidebands will be resonant, then the main peak, then the other sideband. Similarly, if one is sweeping the cavity length, one of the sidebands will first be resonant, then the main band, then the other sideband. In either case, one obtains a scope reading similar to that shown.

Figure 4.1: The cavity sweeps through resonance.
in Fig. 4.2. Then one can scale the time easily using

\[ \Delta \nu_{FWHM} = \frac{\Delta t_{FWHM}(2\Delta \nu)}{\Delta t}, \]  

(4.1)

where \( \Delta t \) is the time difference between the occurrence of the first and second sidebands.

In addition to the sweep apparatus, the laser, and the add sidebands, several other pieces of equipment are needed in this experiment. The following subsections describe them in detail.

### 4.1 Optical Setup

Fig. 4.3 shows the optical circuit used in the experiment. The beam is produced by a titanium sapphire tunable single-mode laser\(^1\). The laser cavity is isolated from the rest of the optical circuit through the Faraday-effect optical isolator. This component is of great practical importance: without it, back-reflection into the laser cavity can destabilize the laser output intensity. The next element is a electro/optic frequency modulator which adds the sidebands at a frequency \( \Delta \nu \) produced by a local oscillator running at between 1 and 150 MHz (see section 4.1.4).

After this stage, the beam is focused into the resonance cavity with a suitable focusing lens. The reflected beam is captured by a photodetector.

\(^1\) Microlase Optical Systems: Single Frequency Ti-Saphire laser, Model MBR 110.
Figure 4.3: Optical setup.
The detector’s AC output is demodulated with a mixer coupled to the local oscillator and then sent through a low-pass filter. Both the DC and the demodulated AC signals are displayed on the oscilloscope.

We will now describe each part of the optical circuit in detail.

4.1.1 Resonance Cavity Design

The cavity cell, sketched in Fig. 4.4, was designed to meet several requirements:

1. The cavity must be short.

To study small numbers BChl$\alpha$ molecules in the beam, the volume of the beam must be small. From the equation for cavity volume (Eq. 4.5),

\[ V = \pi L \frac{\lambda}{2 \pi} \sqrt{L(2\rho - L)} \]

we see that the volume depends most strongly on the cavity length. Thus, a primary design objective was to keep the cavity short. There are several reasons for this requirement. First, the absorption of the cavity without BChl$\alpha$ must be minimized. This requires a short cavity since the absorptive loss due to acetone increases in proportion with the cavity length. Second, the number of BChl$\alpha$ molecules inside the beam volume must be small. This could be achieved with a very dilute solution, but working with very low concentrations is impractical. Thirdly, the response time of the cavity is smaller for a short cavity length. This last point can be seen with a quick calculation. Consider the intensity $I_{\text{circ}}$ inside the beam. If suddenly the input beam is turned off, this intensity rings down according to

\[ \dot{I}_{\text{circ}} = -\frac{I_{\text{circ}} \delta c}{2L} \delta c' \]

where $\delta c$ is the total loss coefficient and $c' = c/n^c$, with $n^c$ the index of refraction. The solution to this equation is

\[ I_{\text{circ}} = I_{\text{circ}(0)} e^{-t/\tau} \]

with

\[ \tau = \frac{2L}{\delta c'}. \]

For a cavity length of 500$\mu$m with a finesse of about 40,000, $\tau \approx 30$ ns in acetone and $\approx 20$ ns in air.

The finished cavity has an adjustable length of 3 mm to 130$\mu$m. Its length can be adjusted roughly through screws which push the PZT and far mirror towards the first
mirror (right side of Fig. 4.4.) This first adjustment is accurate to within about 500 microns. Fine adjustment is done by applying a biasing voltage to the PZT. If mirrors with a focal length of $\rho = 5$ cm are used and the mirror separation is adjusted to $L = 500 \mu m$, the beam waist is only

$$w_0 = \sqrt{\frac{\lambda}{2\pi}} \sqrt{L(2\rho - L)} \quad (4.4)$$

$$= 24 \mu m,$$

giving a beam volume of

$$V_{cav} = \pi w_0^2 L$$

$$= \pi L \frac{\lambda}{2\pi} \sqrt{L(2\rho - L)} \quad (4.5)$$

$$= 1.4nL$$

2. The cell must be solvent tight.
Acetone is a solvent that attacks most polymers. Therefore, the cell is made mainly from steel vacuum parts. One side has a glass window through which the incident and reflected beams pass. The back-side of the cell consists of an elastic membrane made from Kalrez, a perfluoroelastomer elastomer. This membrane is necessary so that the mirror spacing $L$ can be changed with the help of a PZT, which is on the other side of the membrane (see Fig. 4.4).

3. **Liquid exchange in the cell must be easy.** A final requirement is that it must be easy to move acetone and BChl$\alpha$ solution into and out of the cavity. This is done with two access holes and a division in the cell that directs the fluid flow through the mirrors. It is also necessary to make sure that no acetone is trapped in the cell because it can take days to evaporate out. To allow faster run-off, holes were drilled into mirror mount C1.

### 4.1.2 Scanning the cavity

As mentioned earlier, the resonance peaks can be obtained by either scanning the cavity length or the laser frequency. Scanning the cavity is required when one wants to scan over an entire free spectral range (for $L = 500\mu$m, $\Delta\nu_{FSR} = 300$ GHz) since it is much larger than the maximum scanning frequency range permitted by the laser (40 GHz). This requires changing the cavity length by $\lambda/2$. However, scanning the cavity is less stable than scanning the laser. Thus, when one only wants to scan across the resonance peak, which is shorter than the free spectral range by a factor of $F$, one can use the laser to scan instead. Sometimes, one might want to use both methods by moving $L$ close to the resonance length $k\lambda/2$, and then using the laser to scan over resonance. We’ll describe each method below.

1. **Scanning the cavity using a PZT**

Two different piezos (PZT-based) were used in these experiments. The first one was a hard PZT which required a voltage difference of 300 V to move approximately 1 $\mu$m. An amplifier circuit was built to supply a voltage ramp of 0-300V at a frequency of 0-50 Hz. This circuit is shown in Fig. 4.5. This circuit’s output voltage was given by

$$V_{out} = V_0 - \frac{R_0}{R_i}(V_{in} - 0.6V), \quad (4.6)$$
Figure 4.5: High Voltage Amplifier.

with a gain of

\[ g = \frac{R_0}{R_i}. \]  

(4.7)

From Fig. 4.5, is easy to see that

\[ \frac{V_b}{1+g} < V_{out} < V_b. \]  

(4.8)

The input signal was generated by a function generator\(^2\). It varied between 0 and 10 V at the maximum, so a gain of at least \( g \approx 30 \) had to be ensured so that the PZT voltage varied between 10V and 300 V. It is crucial to choose the resistors so that enough charge reaches the PZT during one cycle. Roughly, one should make sure that

\[ R_{max} \ll \frac{1}{4f_{sweep}C}, \]  

(4.9)

where \( f_{sweep} \) is the sweep frequency of the PZT. The hard PZT has a capacitance of 9 nF, so \( R_{0}^{max} \approx 500k\Omega \). To ensure that the amplifier works well, one must choose \( R_0 \leq 0.1R_{0}^{max} = 50k\Omega \).

However, with \( R_0 \) this small, the current becomes appreciable, reaching up to 6 mA. This deposits an average power of up to about 1 W in the circuit. Power this high requires high-power circuit components and heat sinks.

Using the hard PZT, the length change of the cavity was only 300±50µm. This is less than \( \lambda/2 \), so the piezo scanned over less than one free spectral range. The PZT did

\(^2\)BK Precision, Inc.: 20 MHz Sweep/Function Generator, Model 4040
move by more than $\lambda/2$, though. In fact, the interferometer setup (Sec. 4.1.3) showed that it traversed about 500nm when the Kalrez membrane was left out. Thus, some of the motion of the PZT was lost in compressing the Kalrez membrane. I estimate that the Kalrez membrane absorbed about 60% of the PZT’s motion, or about 300 nm.

To solve this problem, one could have either used a thinner Kalrez membrane or farther-scanning PZT. We chose the second option and purchased a PZT stack. The stack consisted of an array of planar PZT layers which allowed it to scan further than single-component PZT’s. When the stack was swept from 0V to 150V (the maximum permissible voltage), the cavity moved through 13 free spectral ranges at $\lambda \approx 800$ nm. By modeling the steel spring and the Kalrez membrane as a system of two springs in series, it is easy to see that the PZT was actually extending over $13 \cdot \lambda/2 \cdot (1 + 0.6) \approx 8 \mu m$. Over time, the PZT degraded and most of the data was taken when the PZT was extending over only about four free spectral ranges, i.e., over $4 \cdot \lambda/2 \cdot (1 + 0.6) \approx 2.6 \mu m$.

The soft PZT was scanned in two ways. First, for the purpose of aligning the cavity, the PZT was scanned over more than one free spectral range. This was done using a signal generator$^3$, amplified by a high-voltage amplifier$^4$. However, the amplified signal was rather noisy, so the resonance peak was unstable. The solution was to sweep the PZT using the function generator added to a battery-supplied biasing voltage. The 300V-battery supplied a much quieter voltage than the amplifier did. In addition, the battery acted as a high pass filter in the circuit, so that most noise from the function generator was suppressed. The function generator signal had an amplitude as high as 5V which moved the cavity through about one-third of a free spectral range. Then the biasing voltage only needed to be changed with a variable resistor to bring the cavity near the resonance length. The 5V ramp could then sweep the cavity across resonance. In this configuration, the sweep had very low noise and the resonance peak was quite stable.

2. Scanning the Cavity using the laser

Instead of scanning the cavity length, one can scan the input frequency of the laser.

---
$^3$BK Precision, Inc.: 20 MHz Sweep/Function Generator, Model 4040
$^4$New Focus, Inc. Model number 3211
As mentioned earlier, the laser cannot scan nearly enough to cross one free spectral range, so one must move the mirror close to the resonance condition. This was again done with the high-voltage battery coupled to the voltage divider. When the mirrors were close to the resonance length, the laser then scanned across the resonance peak. I found the laser scan to be very unstable, so I scanned the cavity length instead. However, the instability in the laser signal was more of a temporary problem. Ultimately, it would almost certainly be preferable to scan the laser instead of the PZT.

4.1.3 Interferometer

The optical setup can be easily transformed into an interferometer setup that monitors the movement of the second mirror (M2). To that end, the first mirror (M1) is removed (Fig. 4.4.) A different mirror is added to interfere the reflected beam with the secondary beam from the non-polarizing beam splitter (see Fig. 4.3). This configuration is useful for determining how the PZT is scanning, and how it is moving mirror mount C2. For instance, in Fig. 4.6, the intensity of the interfered signal was plotted together with the ramp voltage. At certain points, this intensity jumped because of slip-stick friction between the mirror mount and the cavity casing. The interferometer setup is also useful for testing how much the cavity vibrates and if it needs to be mechanically isolated further.
4.1.4 Sidebands

Sidebands of frequency $\Delta \nu$ are introduced using an electro-optic frequency modulator\(^5\). This device relies on the Pockels effect, the linear relationship between the refractive index of a crystal and the electric field applied to the crystal\(^9\). The sidebands are deflected at a very small angle. However, this slight deflection was found to be negligible because the distance between the cavity and the E/O is only about 1m and because the cavity is not too sensitive to small angular misalignments (see Sec. 3.1.1). The E/O was driven with a voltage to frequency converter circuit\(^6\), coupled to a high-frequency amplifier\(^7\).

4.1.5 Detecting the Resonance Peak

If one scans over a free spectral range with a reasonable sweep rate, the peak comes and vanishes so quickly that the photodetector might be too slow to resolve it. For example, if we are sweeping the cavity at 10 Hz over a fraction $\alpha_{FSR}$ of a FSR, and assuming the cavity finesse is 40,000, then the time spent in resonance is merely

$$\Delta t_{FWHM} \approx \frac{1}{4\alpha_{FSR} F f_{sweep}},$$

where the factor of 4 appears because the cavity sweeps through the full spectral range in one-fourth of one period. This evaluates to about 0.6 $\mu$s for $\alpha = 1, F = 40,000, f_{sweep} = 10$Hz. Thus, to get any decent resolution of the resonance peak, the photodetector’s time resolution should at least about ten times better than $(\Delta t_{FWHM})^{-1} \approx 1.6$ MHz, or at least about 16 MHz.

I used two photodetectors. The fast one had a resolution of 125 MHz and was fast enough to detect the resonance peak under the conditions described above\(^8\).

The resonance was seen from the DC signal. However, this signal was rather noisy, so it was often difficult to see the sidebands. Luckily, the AC signal could be used in such cases. It was mixed with the local oscillator signal to demodulate it. Then it was passed through a low-pass filter which let through only the signal we’re interested it.

To see this quantitatively, consider the frequency-modulated incident beam with electric

\(^5\)New Focus, Inc.: IR Resonance Phase Modulator, Model 4003.
\(^6\)Mini-CircuitS 15542, Model No. ZOS-150
\(^7\)Mini-CircuitS 15542, Model No. ZHL-1-2W
\(^8\)New Focus, Inc.: Visible DC-125 MHz Low Noise Photodetector, Model 1801.
Figure 4.7: Transmissivity (in %) of mirrors.

It can be shown from Eq. 3.11 that the reflected beam intensity is approximately

\[
I_{\text{ref}} = I_c |F(\omega)|^2 + I_s (|F(\omega + \Delta \nu)|^2 + |F(\omega - \Delta \nu)|^2) \\
+ 2\sqrt{I_c I_s} (\Re[F(\omega)F^*(\omega + \Delta \nu) - F^*(\omega)F(\omega - \Delta \nu)] \cos \Delta \nu t \\
+ \Im[F(\omega)F^*(\omega + \Delta \nu) - F^*(\omega)F(\omega - \Delta \nu)] \sin \Delta \nu t \\
+ (2\Delta \nu \text{terms})
\]  

(4.12)

If this signal is mixed with the local oscillator signal \( V_0 \sin(\Delta \nu t + \phi_{LO}) \) while the length of the cable is chosen such that \( \phi_{LO} \approx 0 \), then the \( \sin \Delta \nu t \) Fourier component is extracted after the signal is fed through a low-pass filter. This signal is shown in 4.2.

The surface area of the 125Mhz detector is very small (0.8mm by 0.8mm) to allow for its fast response. This small area makes it impractical for use during alignment, where the beam position often changes. For that reason, I used another photodetector\(^9\) with a larger active area of 3.6 mm by 3.6 mm. With a resolving power of 10 MHz, this photodetector is not fast enough to detect on a timescale of \( \Delta_{FWHM} \) when \( \alpha = 1, F = 40,000, f_{\text{sweep}} = 10\text{Hz} \). However, one can decrease the finesse of the mirrors by using an input beam whose frequency is outside of the mirror’s reflective range (see Fig. 4.7). At this point, the finesse might be as little as 100. As is seen from Eq.4.10, the slower photodetector is then more than adequate.

\(^9\)Thorlabs, Inc.: Switchable Gain, Amplified Silicon Detector, Model PDA 55
Figure 4.8: The lower signal shows the sweep voltage to the piezo. As it decreases the cavity length, the resonance peak is overly narrow. As it expands the cavity, the peak is unusually broad.

4.1.6 Resonance Cavity Characterization

A few remarks about the properties of the resonance cavity.

- **Alignment**
  With the current setup, it is straightforward to align the input beam to achieve a coupling efficiency of $\eta_c \approx 1/2$ to $\eta_c \approx 1/8$ into the lowest-order gaussian mode.

- **Heating**
  The heating effect is visible in Fig. 4.8. When the cavity is expanding, the resonance peak is broader than when the cavity is contracting. This happens because as the cavity sweeps through resonance, it transmits a significant fraction of the incident light onto the brass mirror mount C2 (Fig. 4.4). This heat tends to expand the cavity. Thus, if the cavity sweep is expanding the cavity, this heating effect tends to counteract that motion. The cavity then spends a longer time near resonance. This in turn leads to even more heating. At some point, the heating-induced shortening can no longer balance the expansion and the cavity quickly leaves resonance. Thus, as the cavity is expanded by the PZT, the absorption peak is overly broad. This scenario is particularly clear in Fig. 4.9. On the other hand, if the sweep is shortening the cavity as it crosses resonance, the heating effect will tend to contract the cavity even faster. The resonance peak is then overly narrow.

Since the brass mount C2 has much lower reflectivity than the mirror, it eventually absorbs almost all of the transmitted light. Most of the thermal energy then diffuses, though some is radiated off back into the cavity.
To check if this explanation for the strange behavior of Fig. 4.8 makes sense, we can do a few rough calculations. First, we can estimate if the heat that reaches the mirror mount can actually expand that piece sufficiently to counteract the expansion of the cavity.

The burst of transmitted energy raises the temperature of the brass piece by an amount $\Delta T(t, x)$, where $x$ is the distance into the brass mount. Then, if we assume that the thermal expansion is linear and given by the thermal expansion coefficient $\alpha$, the length change of the brass piece of length $L_x$ is

$$\Delta l = \int_0^{L_x} \alpha \Delta T(t, x) \, dx. \quad (4.13)$$

Also, we know the total amount of heat delivered to the brass piece during one sweep across resonance is

$$\Delta Q = \int_0^{L_x} A c_p \Delta T(t, x) \, dx, \quad (4.14)$$

where $c_p$ is the specific heat at constant pressure and $A$ is the area of the brass mirror mount. Combining the two equations, we obtain

$$\Delta l = \frac{\alpha \Delta Q}{A c_p}. \quad (4.15)$$
The heat $\Delta Q$ transmitted is given by

$$\Delta Q = \Delta t_{FWHM} P_{transmitted} = \Delta t_{FWHM} (\eta_c P_{incident})$$  \hspace{1cm} (4.16)$$

where $\Delta t_{FWHM}$ is given by Eq. 4.10.

If the cavity is swept over one-tenth of a free spectral range at 10Hz, the incident power is $P_{incident} = 1 \text{ mW}$, and the finesse $\mathcal{F} = 40,000$, then the length difference is only $\Delta l \approx 2 \times 10^{-16} \text{ m}$. We need to compare this to the length associated with the $\Delta \nu_{FWHM}$. This length is

$$\Delta x_{FWHM} = \frac{\lambda}{2 \mathcal{F}}$$  \hspace{1cm} (4.17)$$

$$\approx 5 \text{ pm}$$

We see that $\Delta x_{FWHM} \gg \Delta l$. Thus, the expansion of $C2$ cannot be responsible for the problem in Fig. 4.8.

But some heat also goes into heating the air between the mirrors. This heat is

$$\Delta Q_{air} = \alpha \Delta Q,$$  \hspace{1cm} (4.18)$$

where $\alpha << 1$. The air heats up by an amount $\Delta T$, according to

$$\Delta Q_{air} = \Delta T c_p.$$  \hspace{1cm} (4.19)$$

Using $c_p = \frac{5}{2} kN$ for air, which consists mostly of diatomic molecules, we find

$$\Delta T \approx \alpha \cdot 0.01^\circ C.$$  \hspace{1cm} (4.20)$$

Using the ideal gas law $PV = nRT$, we find the ratio of the number densities before and after the heating to be $\frac{n_f}{n_i} = \frac{T_i}{T_f}$. The index of refraction $n^c$ is proportional to the number density, so

$$\frac{n_f^c}{n_i^c} = \frac{n_f}{n_i}.$$  \hspace{1cm} (4.21)$$
Defining $\Delta n^c = n_f^c - n_i^c$, we find

$$\Delta n^c = -\frac{\Delta T}{T_i} n_i^c$$

$$\approx 33 \cdot 10^{-6}.$$ (4.22)

Then finally, the change in the effective length of the cavity,

$$L_{eff} = \frac{L_0}{n^c}$$

(4.23)

can be approximated to

$$\Delta L_{eff} = L_0 \left( \frac{1}{n_f^c} - \frac{1}{n_i^c} \right)$$

$$= L_0 \left( \frac{1}{n_i^c - \Delta n^c} - \frac{1}{n_i^c} \right)$$

$$\approx L_0 \frac{\Delta n^c}{(n_i^c)^2}$$

$$\approx \alpha \cdot 13 \text{nm}$$ (4.24)

Thus, we see that even with $\alpha$ very small ($\approx \frac{1}{1000}$), the length change is appreciable compared to $\Delta x_{FWHM}$. Heating of the air between the mirror can indeed cause the observed effect. The calculation for acetone instead of air between the mirrors gives a similar result.

- **Cavity Equilibrium**

  The discussion on heating suggests that one must (i) minimize the incident power and (ii) sweep over the resonance curve as quickly as the photodetector allows. The first option cannot fully eliminate the problem. If the incident power is too small, the resonance curve disappears in the noise background.

  The second option doesn’t fully fix the problem, either. If one sweeps over the resonance curve too rapidly, the light inside doesn’t have time to reach an equilibrium. If the light inside the cavity cannot be assumed to be in equilibrium, the relationships for resonance are not correct. But even without these equations, one fact can be deduced: the resonance peak will be smaller in amplitude but have the same width. Then the half-max height of the peak will correspond to a larger width. Therefore,
Figure 4.10: The $\Delta \nu_{FWHM}$ is larger if the cavity cannot reach equilibrium.

when one scans the cavity too quickly, the $\Delta \nu_{FWHM}$ will be artificially large. This is illustrated in Fig 4.10.

To ensure equilibrium, the decay time $\tau$ (see Eq. 4.2) must be much smaller than the time spent in resonance, i.e.,

$$\tau \ll \Delta t_{FWHM}.$$  \hfill (4.25)

Consequently, one must pick $\alpha_{FSR}$ and $f_{sweep}$ so that

$$\alpha_{FSR} f_{sweep} \ll \frac{\delta_c}{8 L n_c F}$$ \hfill (4.26)

- **Operation in Air**

  The operation in air is very stable. One rather interesting observation in air is that the peak oscillates along the sweep ramp. That is, the peak might occur at a voltage of, say, 2V during the sweep. Then the peak will slowly creep up the ramp until, after about 3 minutes, it reaches 3V, and starts heading back. The period of these oscillations was observed to be about 6 minutes. These oscillations can happen because the cavity doesn’t sweep linearly: it sweeps further at some voltage ranges ranges than at others. Assume the cavity starts out in some region where the cavity sweeps over 50 $\mu$m. It will heat up slowly through the process described above. To counteract the decrease in effective length of the cavity, the peak will occur at a point in the sweep where the cavity would otherwise be longer, i.e., at a higher voltage. During this migration to a higher voltage, the peak will pass through a region where the sweep occurs over a longer range—over 60 $\mu$m, say. Then the cavity spends less time in resonance, so heating is reduced, which tends to cool the cavity again. This completes
the cycle.

- **Operation in Acetone**

  The cavity behaves similarly in acetone as in air. One major difference, however, is that the amplitude of the resonance curve begins decreases as soon as acetone is introduced. The characteristic decay time (the time it takes to decay to $\frac{1}{e}$ of the initial amplitude) is about $\tau_d \approx 5 \pm 2$ minutes. If acetone is kept running through the cavity, the decay happens much more quickly, with $\tau_d \approx 1 \pm 0.5$ minutes. This observation suggests that something in the acetone either become more absorbing or degrades the mirrors.

  We think that the decay is caused by water from the acetone adhering to the dielectric mirrors surfaces. This water film would decrease the mirror reflectivity. If the acetone is kept stationary between the mirrors, dissolved water must diffuse to the mirrors. In this cause, the build-up happens slowly. On the other hand, if acetone is streamed through the cavity, the convective flow carries water to the mirrors more quickly. This explanation was corroborated by the fact that when acetone from a freshly opened bottle was used, the decay happened more slowly than with older acetone that had already absorbed water from the air.

### 4.2 Cavity Alignment

Aligning the laser beam into the cavity can be time consuming. There are four degrees of freedom, two for the incident angle and two for the position. All four must be roughly correct in order to excite resonances (see Sec. 3.1.1.)

After trying a variety of different methods, we resorted to using a simple laser pointer with $\lambda \approx 530 \pm 20$ nm. This wavelength is far outside the reflective region of the mirrors (see Fig. 4.7.) When the beam is pointed into the cavity, four beams are reflected, see Fig. 4.11. A beam is resonant in the cavity when it is reflected from two opposite, curved surfaces. Thus, one aligns the input beam so that the beams reflected from the curved surfaces are superimposed. Once the incident laser beam is aligned, its path is traced with the beam that needed to be aligned at the outset.

This rough alignment is typically good enough so that one immediately sees resonances. Now one tweaks the input beam until only the lowest-order mode and a few higher-order
modes are left. The higher-order modes persist because the waist of the incident beam is larger than that of the lowest-order mode. To avoid exciting higher-order modes, one needs to mode-match the incident beam. We focus the incident beam with a 5cm convex lens into the cavity interior, about five centimeters away. The lens is mounted on an xyz translation stage for fine adjustments. With the use of this lens, a large fraction ($\eta_c \approx 1/2$ to $\eta_c \approx 1/8$) of the incident light intensity can be coupled to the lowest-order resonant gaussian mode inside the cavity.

4.3 Gas/Fluid Circuit

It was mentioned earlier that an important design requirement is that a solution can be easily introduced into the cell and that the cell can later be cleaned again. This was solved through the use of access valves to and from the cavity. The setup that pumps solution, solvent, or nitrogen to and from the cavity is discussed here. A sketch is shown in Fig.4.12. The pipes are made from stainless steel and have a small 1/8th inch inside diameter to minimize the amount of solution in the pipes.

The circuit allows for three basic modes of operation:

- *Introducing solution of BChla in acetone*
  
The solution is injected using the circuit using a syringe. The advantage of using syringes is that they can be replaced with each new batch of BChla acetone solution. This measure minimizes contamination and uncertainty in the concentration of BChla in acetone.

- *Flushing/Cleaning pipes and cell*
  
  Acetone is pumped from a reservoir through the pipes and the cavity. All pipes that are in contact with BChla solution are cleaned this way. Since the volume of
Figure 4.12: Setup for introducing BChlα and acetone solution and cleaning.

the cavity and pipes is only 2.8 mL, the assembly will be very clean after 50mL or more have been pumped through. Instead of using the pump, the acetone can be pumped through using the pressure of the nitrogen gas. Though the flow rate is not as controlled in this case, it is much faster, and is therefore useful for cleaning the cavity.

- **Drying pipes and cell**

Water from the air can decrease the reflectivity of the mirrors. Therefore, the cavity is dried with nitrogen gas. The nitrogen gas is also used to keep acetone from air, which increases the water concentration in acetone.

The pump relies on a systolic design. Such pumps are available commercially, but most are not built to pump acetone. Acetone requires special tubing that is either teflon-lined or platinum-cured silicone. Both types of tubing are too stiff for most pumps. For that reason, I built a pump with a stepper motor powerful enough to pump liquid through special acetone-resistant platinum-cured tubing (the more common teflon tubing is too stiff for use in a systolic pump).
Chapter 5

Results/Discussion

As mentioned earlier, we find the time duration of a resonance peak, $\Delta t_{FWHM}$, and, using Eq. 4.1, transform this time into $\Delta \nu_{FWHM}$. Then we find the absorption coefficient $\delta_c = \frac{2\pi}{\nu} \frac{\Delta \nu_{FWHM}}{\Delta \nu_{FSR}}$.

A summary of the data is shown in Fig. 5.1. The error bars indicate one standard deviation. The absorption peak of BChl$\alpha$ is apparent near $\lambda \approx 770$ nm. This data shows two statistically different absorption values at many points. For example, near $\lambda \approx 770$ nm, there are points with absorption values near .00020, and just below, there are points with absorption values near .00016. This discrepancy can be seen more clearly when only air and acetone data are plotted in Fig. 5.2. The discrepancy arises because the resonance curve was swept too quickly in the data points with higher $\delta_0$. The data points in the shaded area were all taken with a piezo biasing voltage near 50V, whereas the other points were taken with a biasing voltage near 100 V. The piezo sweeps further near 50V. As detailed in Sec. 4.1.6, this causes the peak to appear overly broad, which causes misleadingly large $\delta_0 = \frac{2\pi}{\Delta \nu_{FSR}} \Delta \nu_{FWHM}$.

This problem was not noticed until rather late, so not all of the data points could be repeated (since each data point takes about an hour). Even with this problem in mind, it was difficult to take data where the sweep was fast enough to avoid the heating problem, but slow enough to ensure that the cavity was always in equilibrium. The natural thing to do would have been to lower the incident power. However, to be above the 1 mV noise floor of the oscilloscope, the incident power had to be at least about 200 $\mu$W. As a result, there was still some appreciable noise of 5% in the data points.

The data set with this additional uncertainty and without the points in the shaded regions is shown in Fig. 5.4. The measurements in air and acetone are very close to the
Figure 5.1: Plot of data collected. The plot shows measurements taken in air (A), in acetone (B), and in acetone + BChl a (C).

Figure 5.2: Measurements for (A) air and (B) acetone. Pairs of data show no significant difference in absorption. The data are in agreement with the average mirror reflectivity values advertised by the manufacturer of the mirrors, c.f. Fig. 5.3.
average reflectivity values reported by the manufacturer of the mirrors (see Figures 5.5 and 5.3). The acetone and air measurements should be roughly independent of wavelengths since their wavelengths are in the flat region of the mirror reflectivity (see Fig. 5.3). The data for air and acetone are plotted again in Fig. 5.5. Linear regression analysis on each set of data gives:

- **Regression line fitted to measurements in air:**

\[
\hat{\delta}_{\text{air}} = a_1 + b_1 \lambda \\
= (0.0048 \pm 0.0019) + ((-6.0 \pm 2.4) \cdot 10^{-6} \frac{1}{nm}) \lambda, \\
\chi^2/(n - 2) = .297
\]  

- **Regression line fitted to measurements in acetone:**

\[
\hat{\delta}_{\text{acetone}} = a_2 + b_2 \lambda \\
= (0.0053 \pm 0.0023) + ((-6.7 \pm 3.0) \cdot 10^{-6} \frac{1}{nm}) \lambda, \\
\chi^2/(n - 2) = .293
\]

where \( \lambda \) is expressed in nm. The first slope has a student t-statistic of \( t_1 = -2.5 \) and the second has \( t_2 = -2.23 \). A hypothesis test with \( H_0 : b_i = 0 \) and \( H_1 : b_i \neq 0 \) gives that both slopes are not significantly different from zero at the \( \alpha = .025 \) level.

The extinction coefficients are calculated from the Beer-Lambert Law (Eq. 2.3), which gives the plot in Fig. 5.6.

The exact values are given in the Table 5.1.

These extinction coefficients must be compared with those derived from the spectropho-
Figure 5.4: Plot of reduced data set. The plot shows measurements taken in air (A), in acetone (B), and in acetone + BChlα (C).

Figure 5.5: Measurements for (A) air and (B) acetone. Pairs of data show no significant difference in absorption. The solid line marks the reflectivity curve of an average mirror, plotted also in Fig. 5.3.

Figure 5.6: Extinction coefficients obtained for BChlα.
Figure 5.7: Extinction coefficients for BChl.a compared to extinction coefficients measured in spectrophotometer experiment given by the solid line (see Fig. 3.3).

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>768.3</th>
<th>770.1</th>
<th>771.5</th>
<th>772.8</th>
<th>773.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε (mM$^{-1}$ cm$^{-1}$)</td>
<td>64 ± 16</td>
<td>70 ± 15</td>
<td>64 ± 14</td>
<td>6 ± 15</td>
<td>0 ± 15</td>
</tr>
</tbody>
</table>

Table 5.1: Extinction coefficients obtained for BChl.a.


tometer experiment (see Fig. 3.3). Both data sets are plotted in Fig. 5.7. The fit is poor. The five data points have a weighted mean $\bar{\epsilon} = \frac{\sum x_i / \sigma_i^2}{\sum 1 / \sigma_i^2} = 41 \pm 35$ mM$^{-1}$ cm$^{-1}$. The spectrophotometer results indicate a slope in the region from $\lambda = 769$nm and $\lambda = 773$nm of approximately $-1 \pm 0.2$ mM$^{-1}$ cm$^{-1}$/nm. Linear regression on the cavity data gives a slope of $-14.4 \pm 3.8$ mM$^{-1}$ cm$^{-1}$/nm with $\chi^2/(n - 3) = 2.4$ which shows insignificant correlation at the $\alpha = .05$ level ($\chi^2_{\alpha=.05,n=3} = 7.82$). A comparison of the absolute values of the extinction coefficients between the cavity-data and the spectrophotometer data is not necessary since both BChl.a samples contained impurities which introduced uncertainty into the concentration of the solution (see Sec. 3.3).

It should be noted that measurements within data sets (with identical parameters such as the piezo biasing voltage) are consistent. Each point in Fig. 5.8 is an average over 100 cavity sweeps. The errors in $\Delta \nu_{FWHM}$ of these averaged data points have a mean of 1.5% and a standard deviation of 0.8 %. This result indicates that if the problems with heating and non-equilibrium are solved, this cavity setup could actually detect as few as 45,000 molecules inside the beam (Sec. 3.2).
Figure 5.8: $\Delta \nu_{FWHM}$ measured within trials have low errors.
Chapter 6

Conclusions

Absorption spectroscopy has several advantages over fluorescence spectroscopy for single-molecule studies. Most importantly, it is less intrusive and avoids shelving of fluorescent states. The major challenge in SMAS is achieving high enough sensitivity for detecting minute absorption signal due to absorbing molecules. We have shown that our new approach of cavity-enhanced absorption spectroscopy, where a Fabry-Perot optical cavity enhances the interaction between photons and molecules (in this case, bacteriochlorophyll-a, BChl a), results in higher sensitivity. Furthermore, we have demonstrated that this technique could be used with molecules in solution, with the possibility for in vitro studies.

This project concerned the first step towards SMAS: the design and operation of a cavity for enhanced absorption spectroscopy. The longer-term goal is to observe conformational changes in BChl a while it is embedded in the cell membrane. In the more distant future, this method could be used to observe, in real time, many types of organic molecules as they undergo conformational changes in vitro. Combined with knowledge obtained through crystallography and direct mutagenesis, this information would greatly improve our understanding of many kinds of organic complexes and the processes in which they take part.

The resonance cavity built in this experiment is functioning well in acetone and air. The total absorption coefficient $\delta_c$ is statistically the same for both media, which confirms that acetone is indeed a suitable solvent for this experiment. In addition, the finesse also agrees with the finesse advertised by mirror manufacturer.

The extinction coefficients measured with the resonance cavity did not show a convincing agreement with those obtained with the spectrophotometer. However, there was also not significant evidence to show that the cavity data are incorrect. The error values for the cavity data must be reduced before a definitive statement can be made. The errors in the
measurements of individual trials were only 1.5±0.8 %, which indicates that the absorption spectrum could be measured on as few as 45,000 molecules. This number could be further suppressed if the cavity length is adjusted below the 450 µm used in this experiment.

The unconvincing agreement for the extinction coefficients results from distortions in the resonance curves due to the competing effects of (i) heating and (ii) non-equilibrium (Sec. 4.1.6 and 4.1.6). It is estimated that far more convincing data can be obtained when these problems are fixed by using a lower incident power. In the future, the sensitivity will be further improved by mode-locking the cavity (e.g., using a Pound-Drever-Hall technique) and increasing output sensitivity with optical heterodyne detection.
Bibliography


