# Two-Photon Fluorescent Microscope Undergrad Team Updates

## 1/10/22-2/1/22

Tiyana and Cass

#### Main Goals and Progress

- Examine general structure for a non-confocal fluorescent microscope, its common elements, and how they work.
- Learning the basic mathematics behind lens functionality, focus, and magnification.
- Building a V1 working design for the microscope and ordering parts
- Considering past data structure and optimization of future data
- Working to automate laser setting adjustments with code
- Familiarity with optical equipment/safety procedures/assembly
- Laser alignment practice with quantitative analysis
- Started analysis of signal/power loss through subsystems of a basic setup

#### General Structure of Microscope & Elements

- **Source (pump)**: 1550 nm
- Optical Fiber
- Collimators
- Dichroic mirrors: laser line mirrors
- Lenses: objective lenses: aspherical lenses
- **Filters**: transmission vs reflection properties
- Mounts: kinematic and static
- Translation stage
- Chopper
- **APD:** Avalanche Photon detector
- **Specimen:** Upconversion Nanoparticles: 980 & 550 nm emission
- Optical cage system, breadboards & optical bench:







log(input)

#### Laser Alignment



- Course alignment with red laser
- Inspection and cleaning of fiber cable tips
- 4 degrees of freedom
- Fine alignment with kinematic mounts
- Still getting the hang of walking the beam
- Best alignment so far produced 2.32 mW back
- Measured 5.15 mW loss through circulator

#### Automating Laser Setting Adjustments

Our goal:

Using Python code to control the keyboard and mouse, so that the computer can enter the input power value we set automatically.

Original steps:

- a. set cursor position
- b. Click
- c. delete previous values
- d. type the new number
- e. hit "enter"
- f. repeat steps above per 30 seconds or 1 min

What we already have: Win32.api package

#### Before we get started

With numpy.linspace, we generated 100 data between 4 and 28 (mW), those are the P in values we chose:

In [1]:	<pre>#generate 100 numbers of Pin import numpy as np Pin=[] Pin=np.linspace(4,28,100) print(Pin)</pre>								
	[ 4.	4.24242424	4.48484848	4.72727273	4.96969697	5.21212121			
	5.45454545	5.6969697	5.93939394	6.18181818	6.42424242	6.66666667			
	6.90909091	7.15151515	7.39393939	7.63636364	7.87878788	8.12121212			
	8.36363636	8.60606061	8.84848485	9.09090909	9.33333333	9.57575758			
	9.81818182	10.06060606	10.3030303	10.54545455	10.78787879	11.03030303			
	11.27272727	11.51515152	11.75757576	12.	12.24242424	12.48484848			
	12.72727273	12.96969697	13.21212121	13.45454545	13.6969697	13.93939394			
	14.18181818	14.42424242	14.66666667	14.90909091	15.15151515	15.39393939			
	15.63636364	15.87878788	16.12121212	16.36363636	16.60606061	16.84848485			
	17.09090909	17.33333333	17.57575758	17.81818182	18.06060606	18.3030303			
	18.54545455	18.78787879	19.03030303	19.27272727	19.51515152	19.75757576			
	20.	20.24242424	20.48484848	20.72727273	20.96969697	21.21212121			
	21.45454545	21.6969697	21.93939394	22.18181818	22.42424242	22.66666667			
	22.90909091	23.15151515	23.39393939	23.63636364	23.87878788	24.12121212			
	24.36363636	24.60606061	24.84848485	25.09090909	25.33333333	25.57575758			
	25.81818182	26.06060606	26.3030303	26.54545455	26.78787879	27.03030303			
	27.27272727	27.51515152	27.75757576	28.	]				



As we saw, because of the reason that the numpy module comes with, the data generated are infinite decimals. So we turned this into txt form so that they look more elegant:

1	4.00
2	4.24
3	4.48
4	4.73
5	4.97
6	5.21
7	5.45
8	5.70
9	5.94
10	6.18
11	6.42
12	6.67
13	6.91
14	7.15
15	7.39
16	7.64
17	7.88
18	8.12
19	8.36
20	8.61
21	8.85
22	9.09
23	9.33
24	9.58
25	9.82
26	10.06
27	10.30

This file can be opened successfully already but can not be read so far. The reason will be talked about later.

#### a. Set cursor position & b. click

in [31]: #Code below is only for one single cycle

#open the software

```
import win32api as wn
import win32con
wn.ShellExecute(0,'open','C:\Program Files (x86)\RIO Tunable Laser Control\RIO Tunable Laser Control.exe','',',1)
# get the size of the screen
from ctypes import windll
import time
width = windll.user32.GetSystemMetrics(0)
height= windll.user32.GetSystemMetrics(1)
print(width, height)
#move the mouse to the "set power" module
wn.SetCursorPos((170,158))
#click it twice (if we only click it once, we can't actually get to the button)
wn.mouse event(win32con.MOUSEEVENTF LEFTDOWN, 170, 158)
time.sleep(0.05)
wn.mouse event(win32con.MOUSEEVENTF LEFTUP, 170, 158)
```

- Use win32api to call the software
- Get the size of the screen so that we can estimate the coordinate of the "set power" bar
- After getting the approximate coordinates, MOVE the mouse there
- "Click" it twice

#### c. delete previous values & e. Hit "enter"

With win32api.keybd\_event, this totally work.

So far we can delete the previous digitals.

#Delete the 4 digitals (and 5 if it looks like 12.48) import win32api win32api.keybd\_event(0x08,0,0,0) win32api.keybd\_event(win32con.KEYEVENTF\_KEYUP,0) win32api.keybd\_event(0x08,0,0,0) win32api.keybd\_event(0x08,0,0,0) win32api.keybd\_event(0x08,0,0,0) win32api.keybd\_event(0x08,0,0,0) win32api.keybd\_event(0x08,0,0,0) win32api.keybd\_event(0x08,0,0,0) win32api.keybd\_event(0x08,0,0,0) win32api.keybd\_event(0x08,0,0,0) win32api.keybd\_event(0x08,0,0,0)

#enter the number from Pin or a

#!!! incompleted yet

#hit enter
#win32api.keybd\_event(0x13,0,0,0)
#win32api.keybd\_event(win32con.KEYEVENTF\_KEYUP,0)

1920 1080

#### Difficulty so far: d. type the new number

We can't use the computer keyboard to copy any element in the original list or txt file now.

Strategies that might work:

- Install pywinauto package
- Install pyautogui package
- (The one I prefer the most) Check if the "RIO tunable laser control" software has a LabVIEW version of the laser driver or source program
- Change the RIO laser to M squared laser. (will definitely work)

#### Other topics of discussion/research

- Immersion objective lenses pros and cons
- Consideration of mirror slide for specimen to enhance collection efficiency
- Aspherical lens vs spherical lens for spherical aberration correction
- Use of Lock-in amplifiers for noise reduction

#### **Zemax Simulations**



### 2/1/22 - 2/21/22 UPDATES

Cass & Tiyani

#### Mirror-Enhanced Fluorescent Microscopy

Mirror-enhanced super-resolution microscopy-Yang 2016

- Replacing microscope slide with mirror
- First-surface mirror with protective SiO2 coating and adjustable thickness
- Constructive interference with a high NA objective can occur within the specimen
- Question: will the 2 photon process be ambiguous with this interference/reflection? Will it make the data unusable because photons that reflect may not reflect in pairs causing an energy transfer upconversion specifically as the emission process?
- This was done with confocal microscope with oil immersion objective (NA 1.4)
- Doubled signal intensity
- Emission wavelength ranged from 470-670 nm
- Resolution: ~19 nm
- Mirror-enhanced super-resolution microscopy | Light: Science & Applications (nature.com)

#### Understanding Upconversion Nanoparticles (UCNP)

- About 1- 100 nm in size
- Ideal for high surface area to volume ratio
- Can bond proteins on outside of particle which bond to different structures
- 2-25 % doping
- Effects similar to operations in bulk semiconductors
- Cross relaxation
- Energy transfer upconversion
- Role of crystal lattice

\*\*\*Related:

Experimentally obtained (by chemist team) lifetime of nanoparticles was far longer than expected or thought possible. Thoughts to redo this under more controlled conditions

#### Lock-in Amplifier

- Goal is to calibrate
- Overloads and need to find base overload point and then attenuate it appropriately to make sure it does not overload for our scope
  - Max input voltage
- Did not get very far with this (yet)
- Need to read manual to gain deeper understanding of basic controls

#### **Testing FemtoWatt Detector**

- Oscillatory behavior found when detected power drops below threshold
- Tested with variable attenuator but could not quantify
   turns not repeatable
- Best results for attenuation was slightly uncoupled in tandem with power control from laser source
- Completed initial noise analysis with single trace before and after oscillatory behavior (POST ITS)
- Found 1 Mega Ohm seemed to stop the oscillatory behavior altogether
  - Jan found part that may solve this problem as we only have 50 MOhm for the actual experiment currently
- Research into Fourier Transform theory



#### Brief Summary of Noise Analysis on FW Detector

Noise floor (fft) with 50 ohm (oscillatory behavior cut out) :



Noise floor (fft) with 1 MOhm :



#### Hardware update

- After some nosing around found a shop to cut the breadboard into the correct size
- Board is now vertical and ready for assembly of other optical parts
- Laser line mirror & CT1 came in
- Still missing collar for small beam collimator (backorder) but moving toward using other collimator created with lens (1mm focal length)
- Waiting on attachment part

#### Schematic Updated and Dimensioned







#### Action points moving forward

- Figure out how to mount CT1 to board
- Integrate Tiyani's code with Lock-in Detector control code
- Zmax optical simulations
- Beam waist calculations for collimator
- Lock in Detection calibration and overload limit handling
- 1 MOhm configuration plug in
- Lifetime measurement verification

#### 2/20 - 2/26 UPDATES

Hardware updates

• All parts have come in except collimator collar and possibly 1 MOhm coupling device?

Follow up questions from last week

- Lifetime measurements? Leads on verification?
- Zmax Sim? Any interesting results so far? Will we get to play with Zmax?

Still to be done:

- Finish measuring losses by subsystem
- Integrate code with Lock-in detector
- Calibrate lock in detector for overload limits

#### Starting the Building/Alignment/Calibration Process

- Started with pigtail with in-house made collimator, the 45 degree mirror (both in kinematic mounts) and the CT1 without lens at first down to a mirror and back for rough alignment
- Goal was to align and measure loss within system through each part
  - Found that there was too much fluctuation of the power back
  - $\circ$   $\quad$  Found some loose set screws and corrected
  - When using C610-TME-B, found about 12.5% loss (expecting around 5%). Best coupling back was about 7 mW (without lens)
  - Were concerned about back reflection but found fluctuation still present regardless of lens on/off
  - Ultimately decided the fluctuations were coming from the circulator (which won't be a problem in the future)
  - Found that we were not getting the coupling we hoped for and switched collimators a few times.
  - Ended up finding satisfactory coupling with the F260APC-1550 collimator

#### Starting the Building/ Alignment/ Calibration Process

#### NOTEWORTHY STRUGGLES & HOT TIPS

- Initially had trouble aligning because of running out of freedom on one point
   Found fix by adjusting height of collimator itself and resetting kinematics.
- Getting better at finding returning beam and counter-correcting with initial kinematic mount to provide more freedom at points where it is needed
- CT1 without lens proved to be very helpful while aligning straight down
- Loss in the system seemed to vary depending on collimator?
   Ask about this?
- Considering back reflection and phase "noise" with interference

#### Starting the Building/Alignment/Calibration Process

Where things ended

- Saw minimal loss with F260APC-1550 collimator paired with lens when properly aligned.
- Should be able to measure loss more accurately moving forward
- Best coupling so far with lens: 47.4% (13.28 mW back out of 28 mW in)
  - Not sure if beam is going straight down because of position of CT1/ cage mount
  - Still have not mastered walking the beam
  - $\circ$  Only slight increase with lens from ~12 mW without it
- Ready to integrate dichroic mirror?

Measured	losses	SO	far:	
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Collimator + circulator	~13.2 %		
mirror	~3.3%		
550 filter	~3.4%		

#### 2/27/22 - 3/7/22 UPDATES

• Research on Lock in Amplifier



#### Lock-in Amplifier research

Input/raw signal = Vs(t) = (Vx)sin((Ws)t+a)

Reference signal = Vr(t) = (Vr)sin((Wr)t)

a = phase

• Lock-in amplifier mixes/multiplies input signal and reference signal:

 $VsVr = (\frac{1}{2})(Vx)(Vr)[cos[(Ws)t + (Wr)t + a] - cos[(Ws)t-(Wr)t + a]]$ 

- Integrating over many cycles will end up summing to zero
- However if Ws = Wr, then the integration is preserved with the cosine of a constant
- The lock in amplifier in tandem with the chopper accomplishes this, and we are left with only the frequency where the input and the reference are the same
- The end result is the filtering of every frequency except Ws. The effective bandwidth can be incredibly narrow
- 2 channels/mixers in order to handle cases a=90 degrees for cos(90)=0. Second channel operating on cos(a+90). This prevents signal collapse

#### Aligning for the 90 degree outbeam

- Measured 0% loss in dichroic mirror
- Measured 15.6% loss through the objective

**First iteration:** 



#### Second iteration for 90 degree out-beam alignment

• Aligned beams to overlap



#### Alignment detailed procedure & third iteration build



#### Steps moving forward

- Finish alignment setup via procedure
- Follow procedure for alignment steps
- Use camera and detector to characterize alignment and performance
- Attenuate for the FW detector and install
- Integrate lock-in amplifier
  - Look at completing introduction task to lock-in via manual
  - Calibrate lock-in for coupling required and overload limits
- Move forward on data acquisition
## Updates

3/8/22 - 3/27/22

Cassidy Bliss

#### Alignment with updated schematic and procedure

- After rough alignment, found that focus caused translation (1550 beam)
  - Used phosphor camera & software to correct.
    - Attenuated power to not saturate/damage camera
  - Found coma aberration and corrected by bringing beam toward the direction where focus resides
- Went on to back propagate 9080 nm beam
  - Found that dichroic was initially backwards
- Align 980 nm beam to aligned 1550nm by using the flip mirror and 4 degrees of freedom out of the system
- Then align irises to that collinear pair after
  - This proved to be difficult in the free-standing system

# Using the Phosphor Camera to Quantify Alignment through the irises

- Observed where each iris was focusing by closing one while the other was open
  - Found that they were not focusing in the same place
  - Made adjustments to the positions of the irises (NOT THE BEAM)
  - Got video of airy discs and focusing of each iris

### Cage Mounting and Re-alignment

- New parts came in to replace current mounts etc. for cage system replacement.
- Alignment was a little simpler with this setup
- Found that 1550 and 980 beams had different focal lengths
  - Spoke about divergence of the 1550 collimator and possibly replacing
- Took separate data with more data points to examine full width half maximum vs position of lens
  - Originally completed with both beams instead of individually
  - Re-took data and video of alignment with irises.
  - Used Image J software to calculate from image snapshots at varying positions

### **Results from Data**



- Virtually the same results as Jan
- Focusing at different points but may not be an issue?
- Data points every 5 thousandths of an inch

#### Normal Distribution and FWHM

- Understanding the math and reasoning behind using FWHM to represent the beam
- Derivation of sigma value from Image J analysis from normal distribution of gaussian fit
- Relationship between FWHM and sigma



#### Aligning the Forward Propagating 980 nm beam

• Added third collimator and aligned forward propagating beam to iris position (representing current alignment). Had trouble with clipping on iris



# Updates 3/28-4/28

### Understanding Theory Behind Imaging Code



 Delay between stage acceleration and acquisition markers

 Ensures stage moving at stable velocity through actual data acquisition

#### **Understanding Theory Behind Imaging**

1 Sweep



#### **Understanding Theory Behind Power Sweeps**

- Rio produces 4-28 mW range
- Want a range of 10-28 mW

Data Output columns:	P_in	V_ Out	Std Dev ^2 of V_out	log(std dev) of V_out
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- Plot log(P\_in) vs. log (P\_out)
  - log/log plots good for high dynamic range (data range of dependent variable)
- Can't get all powers at once, must attenuate signal while keeping the 1st sweep non-attenuated, acting as the foundation of the data acquisition
- Can get the 2nd-last sweeps to overlap starting with the "absolute truth" using some proportionality cofactor array.

#### Understanding Powersweep Analysis Goals

- Take average noise measurement (V) for the detector
- Read all data into one matrix
- Create array to store empirical correction
- Subtract noise from all data (arrays in matrix)
- Multiply correction factors through matrix
- Plot with for loop and include error bar iteratively with standard deviation
- Convert to Log/Log plot and error bars to log(std deviation).

#### Measured Loss Through Collimator

Before Fiber Tip	29.6 mW
After Fiber Tip	29.4 mW
Before Collimating Lens	29.4 mW
After Collimator Lens	29.0 mW

Summary in dBm:

Loss Through Fiber Tip	-6.99 dBm
Loss Through Collimator Lens	-3.98 dBm

### **Optimizing Detector Efficiency Through Position**

- Signal optimization with oscilloscope and 1550 nm beam with sample in place.
- Had Trouble with hitting a ceiling and then being out of translation freedom.
- This problem occurred iteratively and when I would try to move the base to allow more freedom, would lose signal completely.
- Found that one problem was that I was starting with too weak of a signal (4 mW).
- Found attenuating with power from RIO was not best

What ended up working:

- Starting with a strong signal and attenuating with the irises
- Box off for finding best base position
- Optimize base position first with translations set to default
- Zoom in to V/division on oscilloscope as maxes hit in each direction.
- Attenuate with both irises as needed, not with power from RIO
- This process took half the time and worked the first time

### Light Shielding Complete

- Build rail standoff from shelf for light shielding
- Sewed blackout curtain to fit rail
- Blackout foil around holes and edges.

(Insert picture here)

### Calibrating Focal Length Shift

 With both 980 nm and 1550 nm beams on, used phosphor camera and highly attenuated power to: (A) do fine alignment on the beam paths, and (B) Correct focal length shift as much as possible.

• Then with varying attenuation, took pictures of both the 980 nm and 1550 nm beam through the same distance at every hundredth of an inch.

#### Notes:

• The back propagating 980 beam required the flip camera to be reinstalled into the system, creating the need for the alignment procedure to be redone.

## Results from image processing on focal length shift

#### FOLLOW UP WITH JAN



#### FW PD Noise Response with new light shielding



#### UCNP Meeting 5/25/22

#### Data Collection from 5 Samples with Current Alignment



#### Individual Slope Analysis of Sample



#### Individual Slope Analysis of Samples



#### Individual Slope Analysis of Samples



Summary of Slope Findings:

Sample	Slope
25% Core@shell (calibration target)	1.811
25% no shell (CU target)	1.1242
IR phosphor	1.8353
25% Paste (no shell)	1.9176
2% Paste (no shell)	1.8516



#### Experimental Setup for Shot Noise Calibration



#### Gain Settings and Offset Setting Analysis



10<sup>^</sup>3 gain setting likely best option

Auto offset may not be a good option for data acquisition

#### **Balanced Detector Laser Noise with Varying Powers**



#### Auto Offset

**Constant Offset** 

#### **Constant Offset Noise Floor with Varying Powers**



Linear Relationship

#### Auto Offset Noise Floor with Varying Powers



Also linear relationship

#### Goals

- Identify proper LO for shot noise calibration measurements
- Verify theory for squeezing factor
- Locate parts for squeezing factor
- Finish squeezed light stabilization
- Make sample measurements with squeezed light

## In Fiber Squeeze Light: Generation and Characterization

**Cassidy Bliss** 

### Introduction to Squeeze (d) E

- Squeezed States of light have noise below the standard quantum limit in one quadrature component
- One property of uncertainty is lowered and consequently the others are increased as per Schrodinger's Uncertainty Principle
- In our case, we generate two-mode squeezed vacuum or twin-beam state (TWB), which means that the intensities of the light beams are correlated with each other at the quantum level.



#### Why are We Using Squeeze light over Classical Source?

- In our UCNP fluorescent microscope, our system is designed around a two-photon upconversion process within the nanoparticles with a 1550 nm light source.
- For biological applications, our concept relies on two photons arriving at the sample at the same time to avoid cellular damage
- We hope to see an enhancement of this upconversion process by using a squeeze light source, increasing our signal to noise ratio, and allowing lower input power with comparable results for imaging purposes without risk of cellular damage.





Photons from squeezed light source

### Degenerate Four-Wave Mixing in Optical Fiber

- Degenerate four-wave mixing (DFWM) is a X<sup>3</sup> (third order nonlinear) process
- Caused by the dependence of refractive index on the intensity of the optical power resulting from the Kerr effect

• Here, the signal and the idler are our squeezed light twin beams



#### Our Squeezing as seen on Spectrum Analyzer



### **Our Squeeze Light Source**

- Seed from Rio at 10 mW
- Two-mode squeezing using fiber as resonance cavity
- SMF 28 as cavity : 1 cm in length
- Thermal control tuning
- Output signal and idler of equal polarizations
- Signal and idler have different frequencies


# **Characterizing Squeeze Light**

1

#### Squeezing Factor (relative to shot noise)

The Squeezing factor gives us the difference between SNL and squeeze light noise (in dBm)

$$S(\Omega) = 1 - \frac{n_c n_d}{1 + \Omega^2 \tau_c^2}$$

 $\Omega = sideband frequency,$ 

 $n_c = 1 - \frac{Q_L}{Q_t}$  = ratio of coupling losses to total losses  $\tau_c = \text{cavity photon lifetime}$  $n_d = \text{the detection efficiency}$  In essence, the squeezing factor will tell us the expected noise loss of the given system, which will tell us how 'well' the squeeze light is being generated as it relates to pairs of entangled photons at the source.

The quality of squeezing quantitatively depends on the ratio between the losses given by the intrinsic quality factor Qi and coupling coefficient determined by the loaded quality factor QL

Experimentally, we can measure the squeezing factor by finding the shot noise level of our system with a classical laser source and then measuring the noise of our squeeze light system

# **Characterizing Squeeze Light**

3

- Stability of Squeeze Light
- A measurement of how much the squeeze light fluctuates in power over a bandwidth



Squeezing Bandwidth

• A measurement of the bandwidth of the squeeze noise that is below the SNL



### Preliminary Theoretical Results: Squeeze Factor

```
%key values
```

```
lambda_seed = 1550.014;
lambda_sig = 1543.10;
W0=2*pi*3*10^8/lambda_seed;
W_offset = 2*pi*3*10^8/lambda_sig;
Qi = 50000000;
Ql = 20000000;
Tc = Ql/W0;
sbf = (W0-W_offset)*(1/6.28);
Qd = 0.85;
```

```
%Key value description:
%Qi = intrinsic quality factor (dimensionless)
%Ql = loaded quality factor (dimensionless)
%Tc = cavity photon lifetime (s)
%sbf = side band frequency (Hz)
%Qd = quantum efficiency of detector (dimensionless)
```

```
%test function/calculate SF:
```

```
x = squeezeCalc(Qi,Ql,Tc,sbf,Qd);
disp('Theoretical Squeeze Factor:')
disp(x)
disp('dBm')
```

```
%Squeeze factor function
function squeezeFactor = squeezeCalc(Qi,Ql,Tc,Qd,sbf)
    Nc = 1-(Ql/Qi);
    squeezeFactor= 1-((Nc*Qd)/(1+(sbf^2*Tc^2)));
end
```

```
Theoretical Squeeze Factor:
3.6504
dBm
```

Published with MATLAB® R2021b

## Experimental Setup for Shot Noise Calibration



# Experimental Setup for Squeeze Noise Measurement



# **Experimental Procedures**

#### Account for Noise Sources and Optimization

- Laser Noise
- FFT Analyzer Noise
- BD Noise
- Optimize and quantify stability of source



#### **Measure SNL**

 Measure the noise floor of the squeeze light system using RIO laser source



#### Measure Squeeze Light Noise

- Filter out the pump and separate the signal and idler with Fiber Bragg Grating
- Mix with Local oscillator at higher frequency reference signal
- Input signal and idler separately into Balanced detector
- Tap into the RF output ESA for noise measurement

# CMRR Updated measurement setup



# New Alignment Calibration Power Results With Optimization:

Input Power (uW)	Output Voltage Previous (mV)	Output Voltage New (mV)
50	800	saturated
10	46	2200
5	14	750
3	5.1	390
2	2.32	200
1	1.15	90

# **MNP** Schematic





# Characterization Of SQL Setup



# Char. of SQL Grating Setup



### Updated Magnetometer Setup



# Ideal SQL signal as seen on oscilloscope for polarization tuning purposes

