Cellular Surgery using Gallium Nitride Nanowires

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Specific Aims

The goal of the proposed project is to design and develop an effective Gallium Nitride Nanowire-based nano-scale laser that, when combined with the improved resolution of Near-field Scanning Optical Microscopy, could serve as a valuable research tool in areas like Stem Cell Research, Neurology, Cell Biology, Cellular Dynamics, and more. Our proposed 'nanosurgery' system could provide a laser spot-size at least an order of magnitude smaller than current technologies, allowing for an unmatched resolution of ~100 nm and less. This tiny resolution is becoming increasingly important as the biological research community continues to probe the nanometer scale. A successful proof-of-concept project would sequentially demonstrate the following specific aims:

Fabricate a GaN Nanowire with lasing capabilities

Improve nano-scale imaging resolution using NW-based Near-field Scanning Optical Microscope (NSOM)

Integrate the NW laser and NSOM into one system capable of manipulation and imaging on the nanometer scale , and experimentally demonstrate the power of such a system

The following project proposal will provide scientific background, significance, and proposed designs and methods to create such a system, as well as some expected results and their possible impact on the greater scientific community.

Background and Significance

Introduction: The field, 'As Is'

The noninvasive study of the biological cell has far reaching research implications in the field of medicine. Understanding the complex interactions and processes that occur at the cellular level provides vital insight into the development and potential treatment of many disorders. Such an understanding would have a significant impact on studies of cellular diseases, including cancer, as well as neuronal and stem cell studies that depend on a detailed knowledge of cell processes. Despite the huge potential that comes with a detailed understanding of cellular systems, inherent difficulties in manipulating and visualizing material at the micro and nano scales remain. In general, such valuable techniques have made limited progress in this field.

Conventional cellular studies have relied on external and chemical manipulation, using micromanipulators and knockout mutants as primary investigative tools [1]. However, advances in the field of optics have pushed lasers into the forefront of molecular and cellular study, as the precise delivery of heat and energy enables lasers to remove or ablate cellular components without compromising the cell's physiological integrity [5-12]. To avoid unintended disruption to the cell, parameters like duration and

intensity of the laser must be carefully determined and oftentimes require lasers with unique physical properties. Recently, femtosecond pulses from titanium sapphire lasers have been focused via standard microscopic lenses to very small spot sizes (~800 nm), and shown to perforate cell membranes, ablate organelles, and cut intracellular filaments [5-12].

Although the ability to remove material at the nano scale is a powerful tool, research involving such a method will need an accurate representation of the effects of changes in the cell. As nanometer scale imaging of biological surfaces becomes a more commonly sought after goal, new techniques and applications of recent technological advances will become more important. Near-Field Scanning Optical Microscopy (NSOM) is a rapidly developing technique in which small scale optical and topographical images are captured by exploiting properties of evanescent waves. These standing waves, which are reflected by the surface of a specimen, are most intense within one third of the wavelength of reflected light, and decay exponentially as distance from the surface increases [2-4]. By placing a detector much closer than the wavelength of the emitted wave, it is possible to capture these waves and in doing so, attain an image of high spatial, spectral and temporal resolution. Operating in the aperture-less mode, the resolution achieved using this technique is subsequently governed primarily by the diameter of the detection element: the NSOM tip. Variations of this technique involving fluorescence imaging have achieved resolution on the nanometer scale, and provide real-time optical and topographical information about the surface that is scanned [3]. However, until recently, manufacturing limitations of the tip size prevented nanoscale NSOM, and as a result, instrumentation remains expensive and relatively difficult to functionalize.

Background and Significance

Neurology

The potential of cellular manipulation is already being explored in many areas of cellular research. The study of neurology is using ultrafast laser pulses to conduct axotomy, or surgical processes on axons. Research groups have targeted axons of tractable organisms, *C. Elegans* for example, that have regenerative properties [7-8]. By studying the regenerative response in simple animals, researchers are working to understand why human neural cells do not regenerate, and to understand the cellular differences that prevent it. Furthermore, Rao et. al investigated intercellular transport in axons by severing connections in a pathway [9]. This assay gives information about axonal transport, and can provide insight on dysfunctional neural networks common in human degenerative neural diseases. *However, greater precision in axotomy would enable a more controlled study of the neural networks formed between large numbers of nerve cells, because connections between cells occur at dendrite junctions, which are nanoscale structures.*

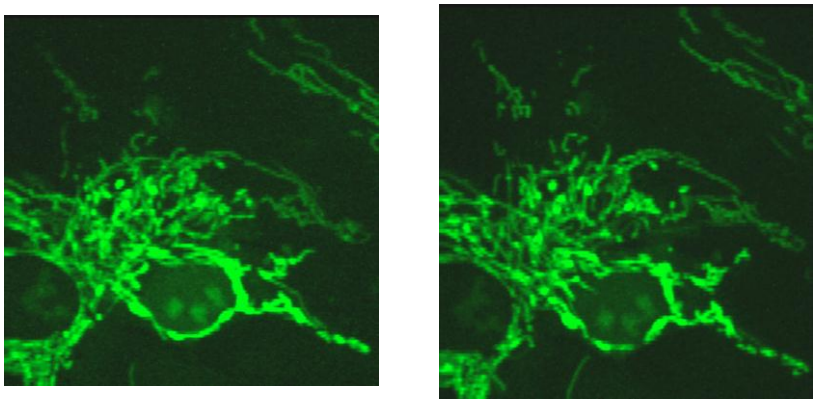

Figure 1. An axon cell, before and after nanosurgery performed with a femtosecond laser.

Cellular Dynamics

Many other fields of cellular study have been impacted by ultrafast laser pulses. In understanding the uncontrolled division of cancer cells, researchers must observe complex malfunctions in chemical and genetic pathways that lead to lethal tumors within the body. This process will be facilitated and enhanced by cellular manipulation, and some groups have begun studying the effects of removing mitochondria from cancerous cells on proliferation of cancerous cells [10-11]. This and other methods of photodynamic therapy could provide information about the processes and abilities of cancer cells, *but further accuracy in these methods would provide researchers insight into the role of specific proteins, hormones, and other nanoscale factors in uncontrolled cell division.*

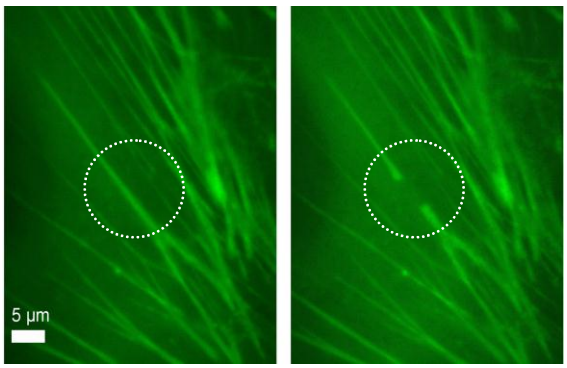

Figure 2. Cellular dynamics studies on cellular fibers, before and after nanosurgery

Stem Cell Research

Stem cells are perhaps the most exciting area of cellular research due to their ability to differentiate into all cell types found within the body. This ability could be

engineered to treat a host of disorders and diseases, and could revolutionize treatment of conventionally untreatable conditions. However, the emergence of stem cells has revealed gaps in our knowledge that must be filled if we are to take advantage of their full potential. More information about the intrinsic controls that keep stem cells at a steady state or direct them along particular differentiation pathways must be attained in order to functionalize the immense capabilities of stem cells. It follows that information about the nucleus of stem cells is of particular interest, as it directs the production and differentiation of the cell through genetic code [13]. Multiple groups have described successful transfection of plasmid vectors to the nucleus of embryonic stem cells, which was induced by perforation of the cell membrane by laser pulses (Figure 3). Other groups have demonstrated laser pulse capability to enucleate cells, facilitating nuclear transplant of autologus nuclei to totipotent stem cells [13-15] (Figure 4). *These and other stem cell studies will be enhanced by nanometer accuracy and non-invasive procedures for cellular modification, and could prove crucial in understanding differentiation and functionalization of stem cells.*

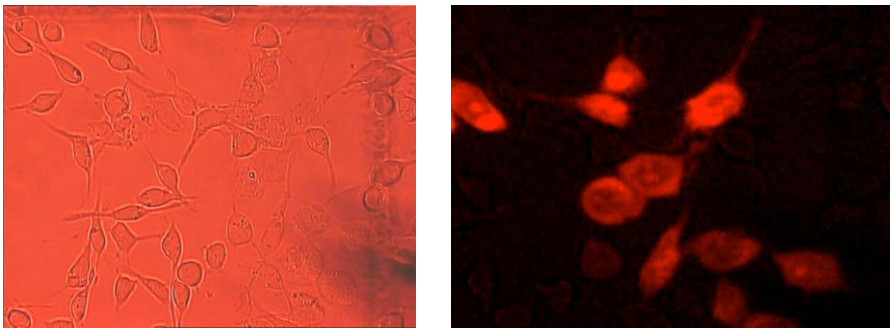

Figure 3. Demonstration of the ability to perform transfection.

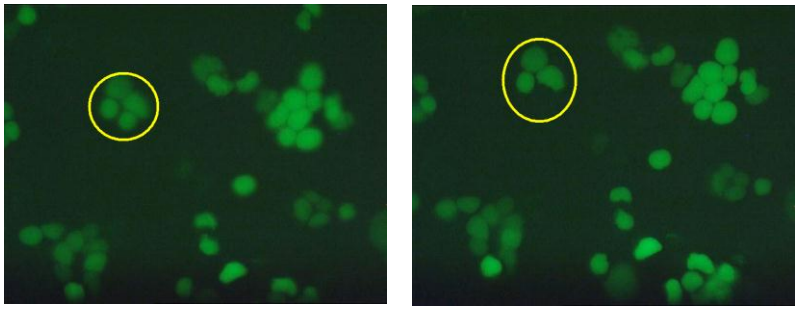

Figure 4. Enucleation of cells, important for Stem Cell Research

Research Design and Objectives

Specific Aim: Fabricate a GaN Nanowire with lasing capabilities

Background: Why Gallium Nitride nanowires?

Gallium Nitride nanowires (GaN NWs) are semiconducting nanometer-scale (10^{-9} m) structures, capable of revolutionizing nano-electromechanical systems (NEMS), high-frequency/high-power optoelectronic devices, water purification systems, photovoltaics and more. Their size (~100 nm dia.), extremely low defect-density, bandgap in the UV (E_g = 3.45eV), high mobility (μ), and high breakdown field make GaN NWs ideal in yet another application: nano-scale ultraviolet lasing.

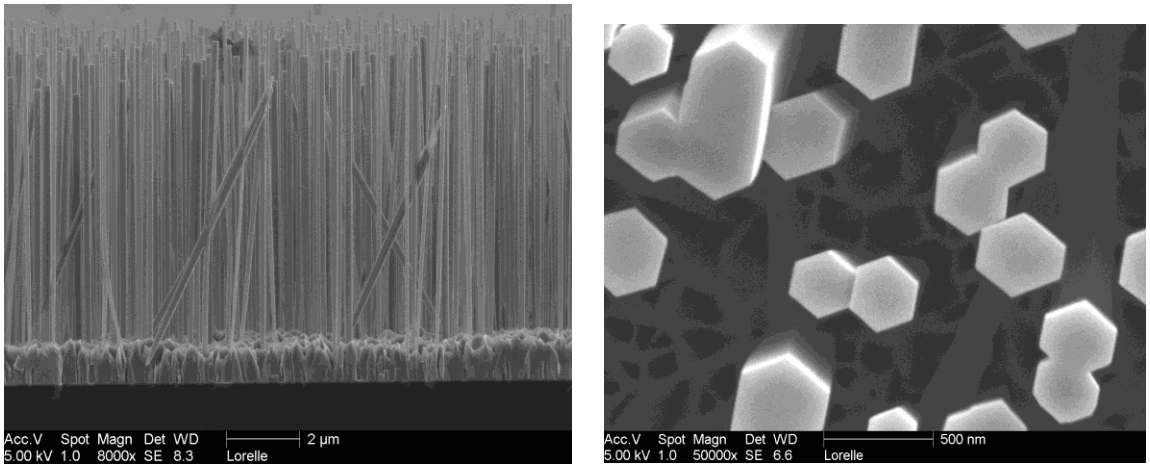

Figure 5. SEM images of c-axis, catalyst free, GaN nanowires grown on $Si/Si0_2$. On the left is a cross section, showing nanowires ~10 μm long. At right is a plan view of the same NWs, showing hexagonal crystal structure and ~100 nm diameter.

A laser (Light Amplification through the Stimulated Emission of Radiation) requires a population inversion between two energy levels in the system, collectively referred to as the bandgap of the material. This can be achieved by optically pumping the material, but for real applications (such as in-vivo targeted UV treatments) population inversion is achieved electrically (Figure 6). This requires electrical contacts to both the n-type and p-type regions of the material.

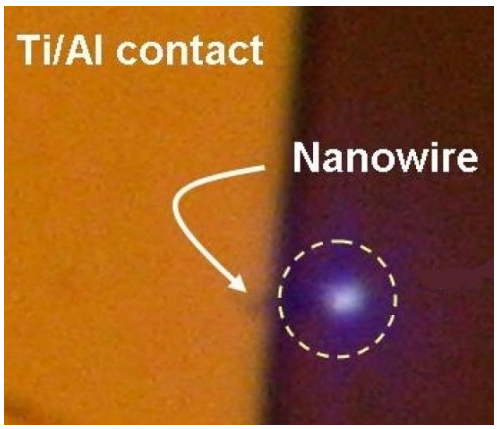

Figure 6. Electroluminescence demonstrated in a GaN nanowire

Proposed Design

We propose fabricating the device shown in Figure 7.

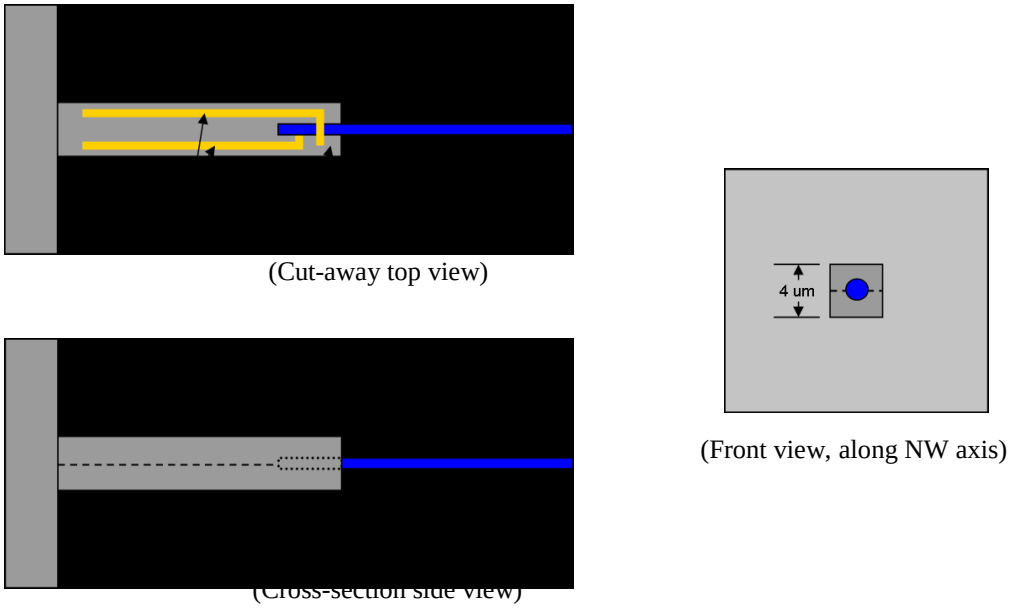

Figure 7. Schematic drawings of proposed NW laser die. The NW is dispersed onto a Si substrate. Metal leads connect both the p-type outer shell and n-type inner core, allowing for electrical stimulation. (features not to scale)

Methods

We begin with an n-type GaN nanowire, uniformly coated (using Atomic Layer Deposition) with a p-type material such as amorphous silicon. Using proven processing techniques (photolithography, metal evaporation, and dielectrophoresis), these coated

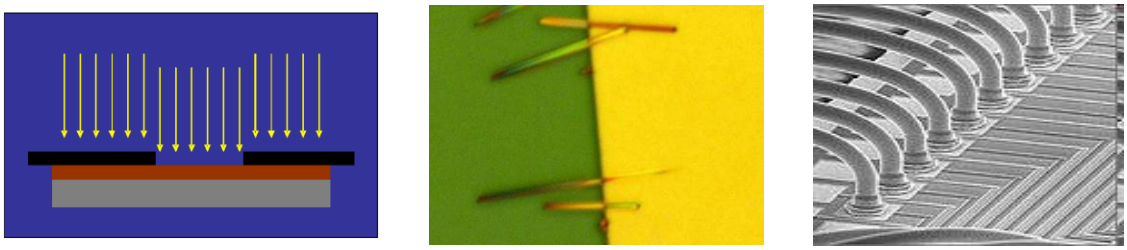

Figure 8. Photolithography, NWs aligned using dielectrophoresis, and wire-bonding

nanowires can be deposited onto a Si substrate. Then, by etching away the over-coating of p-type material, we can contact both regions of the nanowire waveguide, and provide electrical stimulation for lasing. Once electrical contacts are established, the substrate that the nanowire sits on can be etched away using bulk micromachining techniques, leaving only a nanowire laser ‘die’. This nanowire/ substrate/ contact pad die could then

be installed onto a microscopy stand, similar to those housing atomic force microscopy (AFM) or Near-field Scanning Optical Microscopy (NSOM) setups. By increasing the current through the nanowire (via a low current-sensitive external power supply connected to the metal leads deposited on the die), a rapid increase in the light emitted is expected. The wavelength of the light emitted is directly proportional to the bandgap of the material.

$$E \quad \hbar \quad h \quad \frac{hc}{} \tag{1}$$

For GaN, a bandgap of 3.45 eV corresponds to a wavelength of ~365nm. Nanowires have been shown to withstand on the order of hundreds of μA. As a result, we could create an effective nano-scale UV laser that is mounted to a microscope stand.

Specific Aim: Improve nano-scale imaging resolution using NW-based Near-field Scanning Optical Microscope (NSOM)

Proposed Design

As described earlier, the major restriction preventing modern NSOM setups from higher resolution is the size limitation of the NSOM tip. This limitation can be overcome by again using the tiny diameter of a GaN nanowire (~100 nm) to our advantage. We propose modifying the tip of an NSOM by adding a GaN nanowire, which would allow for a much higher resolution image than currently possible. The nanowire would function just like a normal NSOM tip, but would have a diameter at least an order of magnitude smaller, allowing for an even more detailed scan of a surface. Currently, a NW-based NSOM is being developed by a team of scientists at the National Institute of Standards and Technology [16]. They have effectively performed this procedure, and are currently testing the instrument. As a result, this section of our proposal is rather abbreviated. The modified NSOM tip can be seen in Figure 9, below.

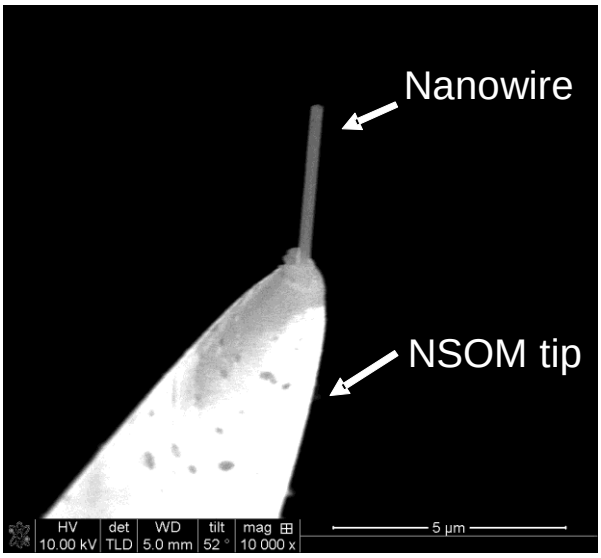

Figure 9. Actual SEM image of a traditional NSOM tip modified with a GaN nanowire using the impressive 'nano-welding' capabilities of a Focused Ion Beam etcher (FIB).

Methods and Procedure

Our design methods would involve simply welding a nanowire to an NSOM tip. This may not seem as simple as is stated, but modern equipment used for nano-characterization can achieve this. Two such instruments are a scanning electron microscope (SEM), and a focused ion beam etcher (FIB). The FIB allows for nano-scale etching, welding, and manipulation, while the SEM provides imaging.

Once a nanowire NSOM tip has been fabricated, we can use proven NSOM techniques to inspect the surface of a sample such as a biological cell. Such techniques include [17]:

- Lightsource: a separate laser focused into an optical fiber through a polarizer, a beam splitter and a coupler. The polarizer and the beam splitter provide filtering to remove stray light from the backscattered light.
- Feedback mechanism and piezoelectric sample stage: to ensure the tip stays within the requisite distance to the sample in order to detect a strong enough signal from the backscattered waves
- Detector: a normal NSOM tip replaced with a GaN nanowire, fabricated as described above, coupled to a standard photomultiplier tube (PMT) or CCD camera.

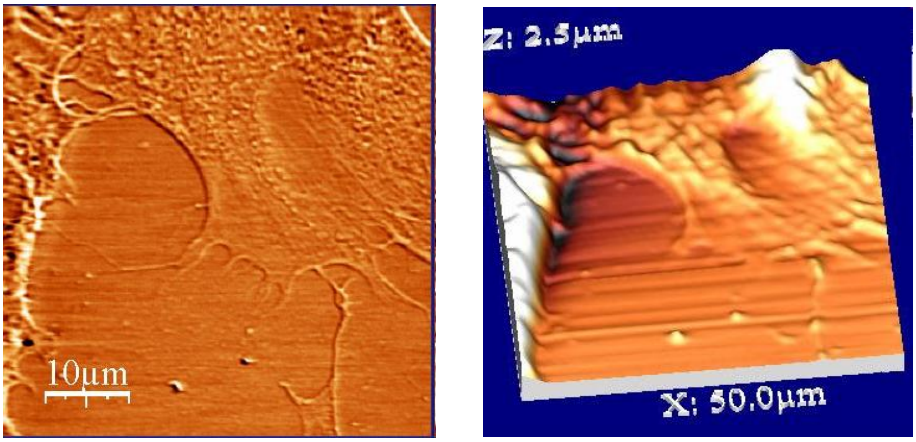

Figure 10. Optical and topographical images (alternate coloring applied) obtained using Near-field Scanning Optical Microscope

Specific Aim: Integrate the NW laser and NSOM into one system capable of manipulating and imaging on the nanometer scale, and experimentally demonstrate the power of such a system

Methods and Procedure

As mentioned earlier, given the integration of our proposed GaN NW laser die into a microscopy stand, and the modification of the NSOM with a GaN NW tip, we can easily combine the two devices into one coherent system. For this experimental setup, we propose essentially retrofitting an existing NSOM setup. The NSOM tip will be slightly modified due to the GaN nanowire, but the NSOM will operate under normal conditions (Figure 11).

To control the GaN NW laser, we propose using a second NSOM setup, slightly modified to accommodate the GaN NW laser. In this way, the displacement-sensitive stage used to control an NSOM tip can be used to also control the GaN NW laser. This setup is desirable for many reasons:

- Nanoscale displacement control in three dimensional space
- Similar reaction times and signal to noise ratio between the NSOM and the NW laser
- Simultaneous nanoscale imaging and operation
- Potential for automation of analysis using relevant analysis hardware and software (spectrometers, LabVIEW or similar programming language, etc.)

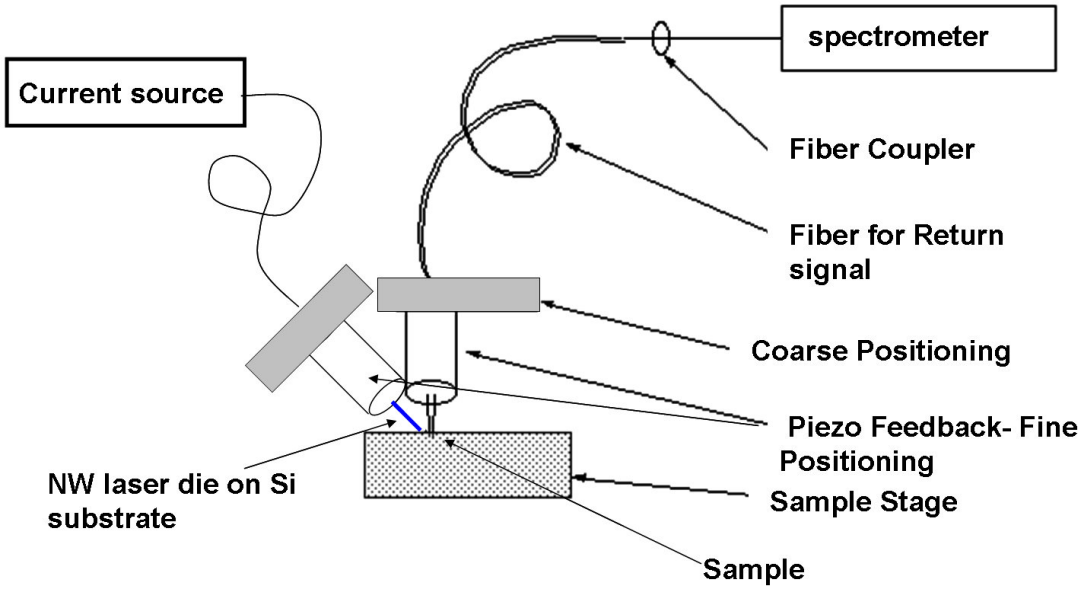

Figure 11. Proposed experimental setup for nanometer scale cellular surgery. GaN NW-based laser is shown on left, and a typical NSOM setup at right.

Procedure

Below we outline an example procedure possible with our proposed system- a transfection procedure common in Stem Cell Research applications. The procedure would proceed roughly as follows, and would vary slightly depending on the ultimate task to be completed:

- Image target surgery area (cellular surface) with NSOM before operation begins to gain knowledge of surroundings and working environment.
- Introduce NW laser to target area, and perforate cell wall with laser pulse.
- Introduce plasmid vectors to the nucleus of embryonic stem cells, using NW laser where necessary.
- Repeatedly image transfected cell(s) to observe changes in the cell as a result of transfection (effectively transmitted DNA survives via mitosis).

If successful, our approach, using the proposed apparatus, would have greater than 70% efficiency (as measured by actual protein expression), and would even be possible in human stem cells because the procedure is less intrusive and less damaging than other technologies currently in use.

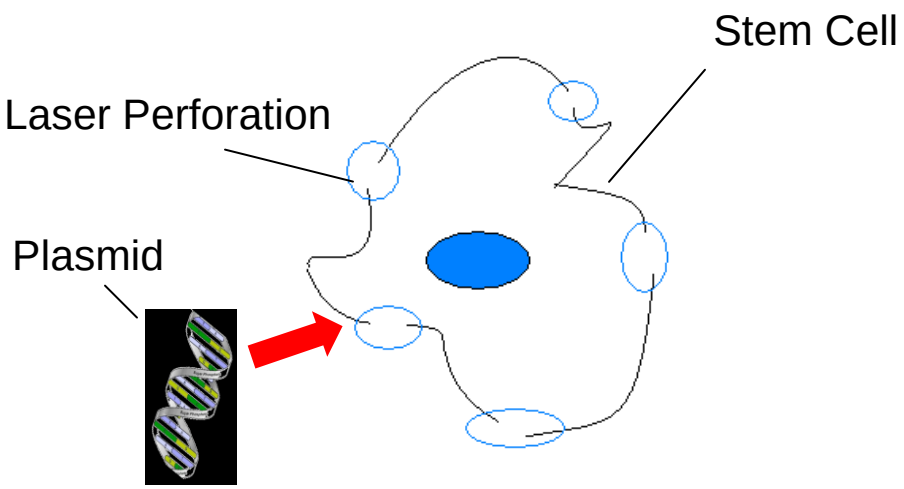

Figure 12. Diagram of transfection procedure, aided by GaN NW-based laser

Potential Experimental Difficulties and their Possible Solutions

As with any experiment, we will certainly have difficulties. Some possible experimental difficulties and their potential solutions are listed below:

System Fabrication

- Precision of nanometer scale fabrication
  - Clearly an issue with anything sub-macro scale
  - Tools like the FIB, SEM, and processes like photolithography, dielectrophoresis, and metal evaporation will limit our difficulties
- P-type contacts to NW
  - Due to lower mobility values and oxide residues and empty surface states, p-type contacts are often difficult to fabricate
  - We hope to employ oxide-cleaning etches and proven p-type contact recipes to ensure ohmic contacts to the p-type region of the nanowire
- Integration of NSOM with GaN NW's
  - Integration has inherent difficulties
  - Fitting everything on the sam sage

System Operation

- GaN nanowire lasing capabilities
  - Electron mobility, E_{br}, and heating are potential issues that could prevent GaN NWs from providing the requisite short laser pulses for operation
  - Growth parameters and doping densities could be tweaked to allow lasing
- Restricting lasing to one specific site
  - Extensive testing of the lasing properties of GaN will be a large part of the experiment
  - Determining the depth and intensity of laser
  - Pulse duration, spot size, and other parameters will be determined experimentally
- Physical rigidity of GaN NWs
  - GaN NW could physically damage cell, if not operated carefully.
  - Other options include the use of CNTs that may be more forgiving (Figure 13)
- Dynamic nature of biological cells
  - NSOM is normally used for surface studies- to study dynamic, soft media carries inherent difficulties in obtaining reliable images and precise operations
  - We hope the sample stage, combined with historical and automated NSOM data will aide in keeping track of the sample and its components to be studied
- Mechanical operation on the nano scale
  - We hope piezo-controlled sample stands will rectify this, as they have for many NSOM experiments.

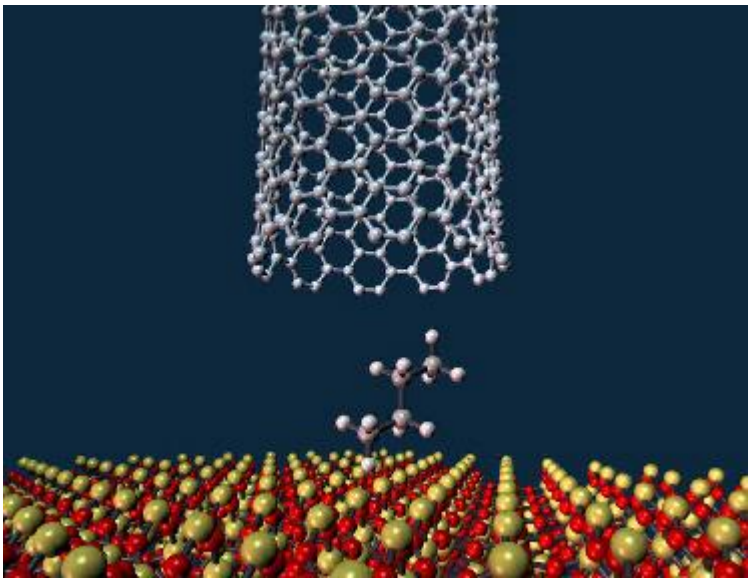

Figure 13. A concept image of a SWNT, with a diameter similar to that of a GaN NW, used as an NSOM tip to probe a butane molecule on a quartz substrate. Possible issues caused by the rigidity of GaN could be overcome by using CNT instead.

Expected Results and Impact on the Field

A successful experimental demonstration of our device would effectively perform one of the research experiments previously mentioned. Following successful completion of a task demonstrated previously (using a different technology), the apparatus could be used in more advanced studies demanding higher precision. There are multiple ways that a device like this could be used, including non-biological applications.

Results from the first phase of our investigation, in which we will evaluate the feasibility and efficiency of Gallium Nitride Nanowires as tools to isolate and remove organelles and genetic material in living cells would have far reaching implications for future research. Real-time feedback provided by NSOM and the precision enabled by using NW's would be revolutionary in the field of cell biology. In addition to the scientific community at large, specific fields of cell biology mentioned earlier in the report would receive immediate and substantial benefit. Furthermore, decreasing the cost of production of such a setup would enable greater diffusion of cellular knowledge and would likely expedite cellular studies. Through proving the potential of improved manipulative surgery at the cellular level, our device could be used for numerous applications in fields of research, and modern medicine.

The ability to operate at an order of magnitude smaller than conventional methods while attaining topographical and optical images of cellular activity in real time would have tremendous impact in the scientific field. Information about smaller and less studied components of the cell involved in protein production and expression could have

far reaching implications in all fields of cell biology. Studying the effect of functional and structural elements of cell bodies that are smaller and less studied components of the cell involved in protein production and expression could shed light on a host of new research, and the impact on existing cellular studies would be similarly significant.

In addition, this procedure should provide a more reliable and efficient substitution for current transfection techniques due to the precision of the device. This process could also lead to more efficient synthesis of functional cells in life-process related fields. For example, mitochondria and chloroplasts have their own DNA due to their endosymbiotic origins and replicate themselves during cellular division in a method similar to binary fission. Using laser surgery, the number of organelles could be regulated to produce specialized cells able to photosynthesize or undergo respiration at a greater rate than would be possible for naturally occurring cells. This would improve the production of proteins or other cellular products, and increase efficiency in pharmaceutical fields where cellular processes are employed.

During the second stage of our proposal, the same methods will be used on stem cells through which the device will theoretically enable accurate documentation of differentiation within the cells as they specialize and provide a greater insight into how the change is triggered. This device could provide a major medical breakthrough in the field of stem cell research and the results of this analysis would help determine whether or not stem cells can be modified both *in vivo* and *in vitro* to generate new tissue. *In* vivo procedures could lead to effective treatments for conditions such as neural diseases which are either currently untreatable or who's treatments are mainly inefficient. *In* vitro processes could also benefit, because any new tissue formed would be perfectly compatible with that of the host's original. This could reduce the reliance on donors in cases requiring transplantation and grafting. Once the abilities and limitations of the device have been determined, research into its potential uses could be widened to cover other important medical fields including degenerative and regenerative neurology, a very active field of study. As a whole, the reduced laser spot size and increased precision would enable and supplement ongoing research, and could prove to be a valuable tool to better understand the processes involved within cells.

These and other unforeseen impacts could be of great benefit to science and potentially humanity, and although device functionality is a difficult undertaking, we feel the benefits are substantial enough to attempt it.

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