Rapid-Test Multiplexed Biochip for Post-TBI Prognostics

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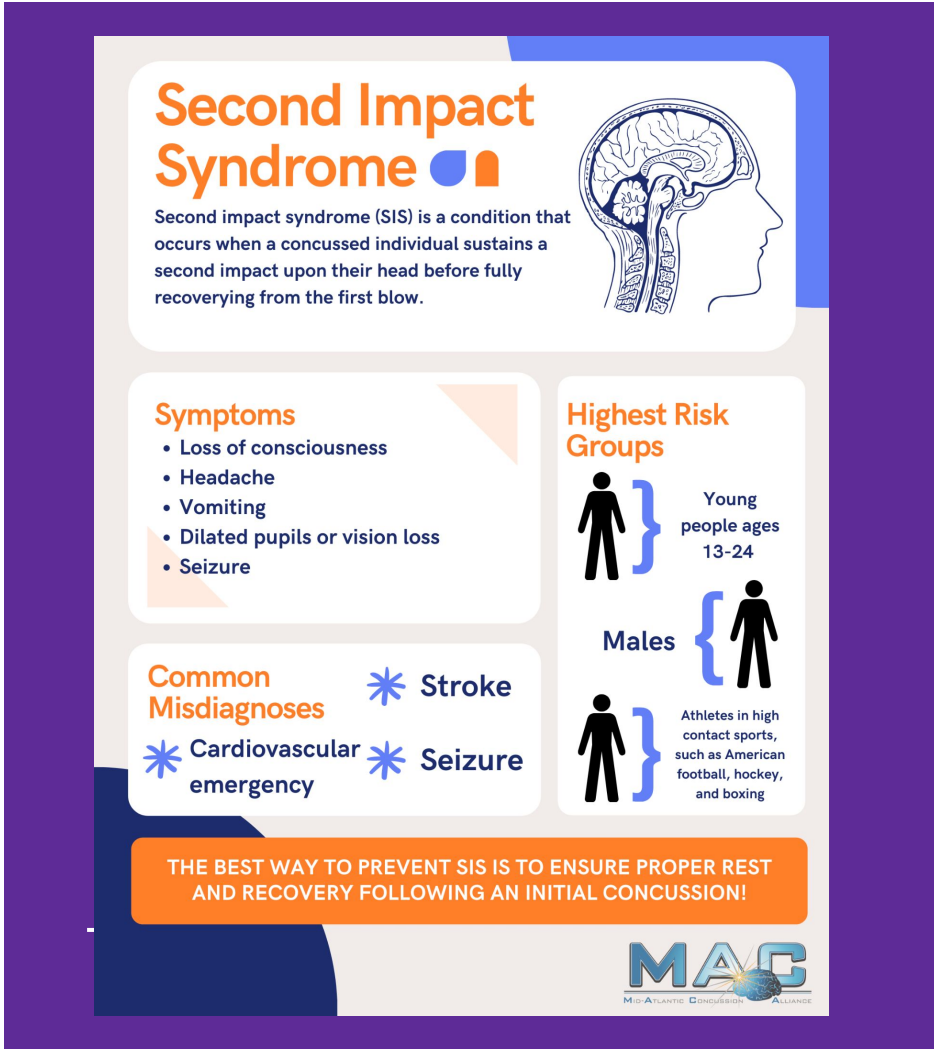
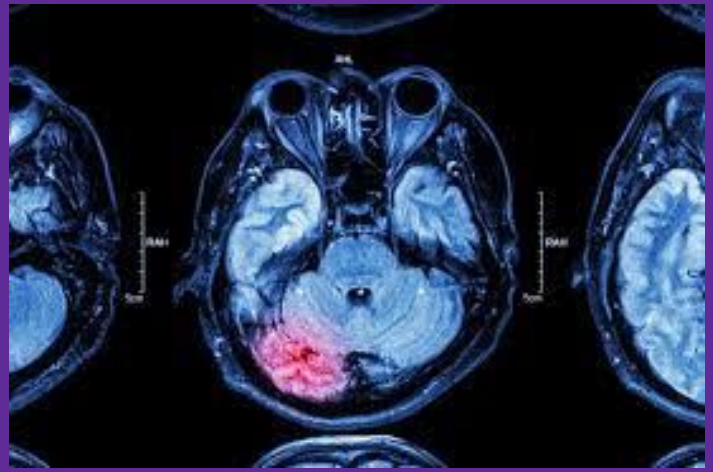
Concussions/TBI

- Bump, blow, or jolt to the head which disrupts the normal functions of the brain
- Range of severity
  - Mild - brief change in mental status
  - Severe - extended period of unconsciousness or memory loss
- Can disrupt development in children
- Often go undiagnosed in adults
- Second Impact Syndrome
  - If symptoms from earlier concussion subside, second concussion can cause a rapid brain swelling



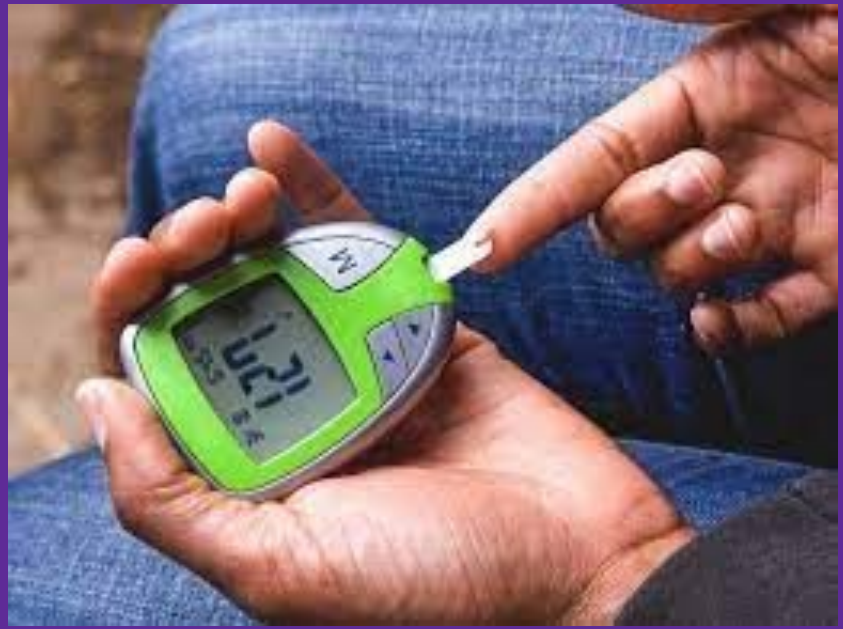
Current Diagnostics

- Glasgow coma scale
  - Physician observes eye opening, verbal response, motor response
  - Quantifies each on 1-5 scale
  - Mild: 13-15 score
  - Moderate: 9-12
  - Severe: <9
- Other neuropsychological assessments
- CT scan, MRI
  - Brain bruising and swelling
- Blood Tests
  - Abbott i-STAT TBI diagnostic tool
  - Plasma extracted from blood via centrifuge
  - UCH-L1 and GFAP measurements
    - Claim a 40% reducal in CT scans needed



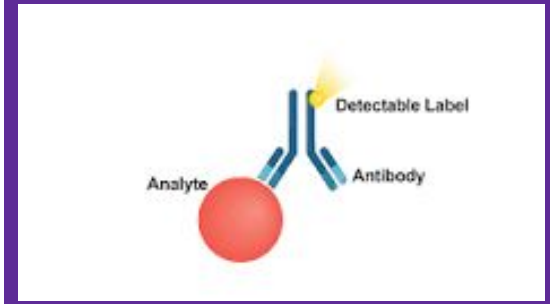
Opportunity

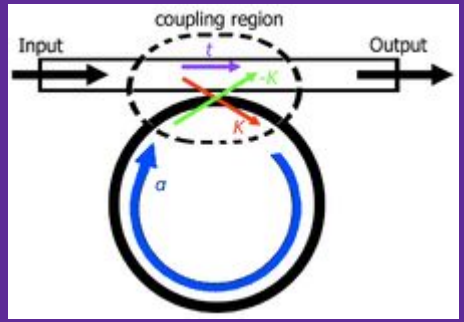
- There are multiple biomarkers that indicate a TBI beyond Abbott's i-Stat (UCH-L1 and GFAP)
  - S100b was the leading indicator in multiple studies which confirmed results with CT scans
- What if we made a product which utilized multiple biomarkers, including S100b?
- Important to monitor patients post-TBI
  - Could use lab on a chip like a finger prick glucose monitor

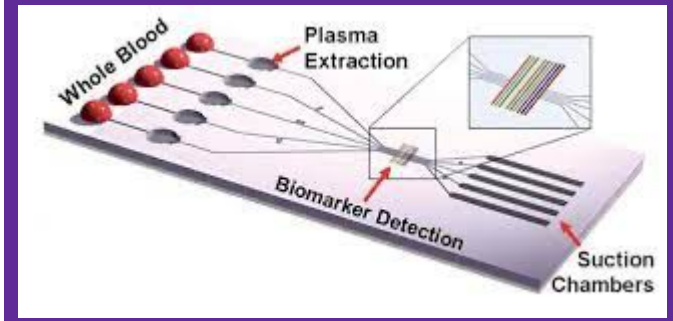


Theory

- Immunoassay allows high specificity labeling of analyte
- Blood can be separated with existing LOC platforms
  - Microcentrifugation
  - Magnetic bead assisted
  - Size-exclusion based
- Optical ring resonance to perform multiplex immunoassays in real time
- Transducers: Convert signal to electrical signal which can be streamed easily for rapid physician evaluation





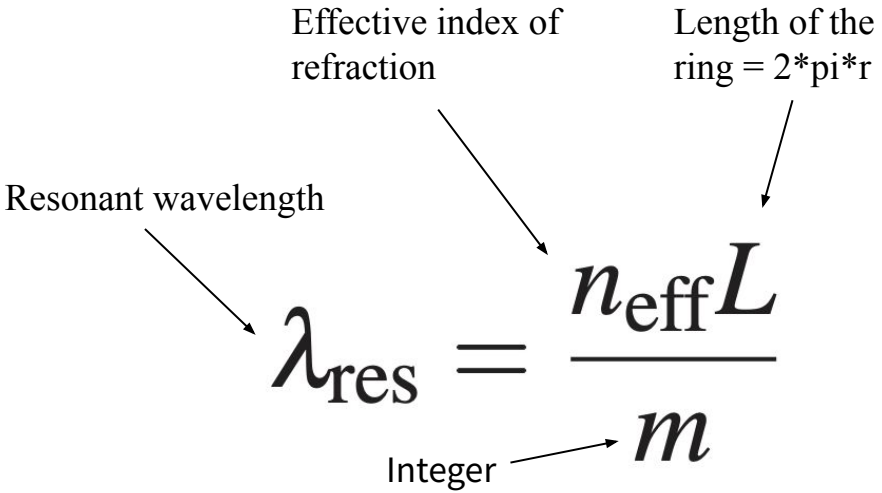


E.g. of a lab on a chip approach to isolating blood plasma

Optical Ring Resonance

Basics

- Use of an optical waveguide
- Total internal reflection (TIR)
- Loops back onto itself
- Resonance occurs when optical path length is an **integer** of wavelengths



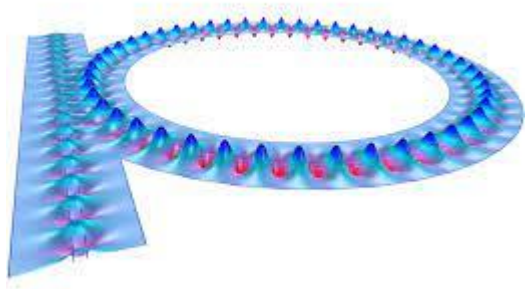
Silicon Based Application

- It's often desirable to have a large FSR —> **need small rings**
- Silicon is an ideal material because it has high refractive index contrast with oxide or air—> allows for extremely small bends
- High sensitivity

Applications for Immunoassays

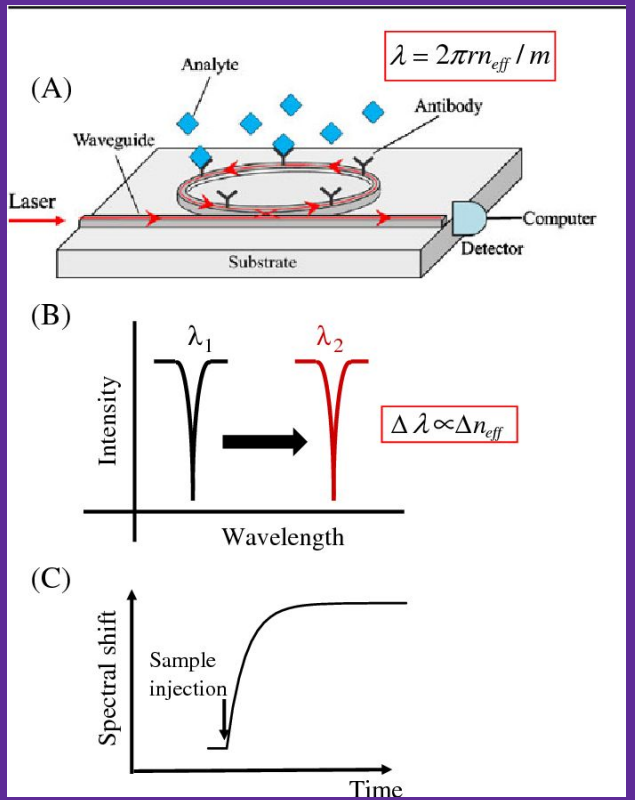
- Creating the waveguide so light energy extends beyond it will make an evanescent trail
- Matter that changes the index of refraction will change the resonant wavelengths —> binding of proteins or DNA can be detected due to their indices of refraction being greater than water (n_{eff})

- Pre-deposit materials onto the rings surface
- Binding of analytes to target displaces water (low n_{eff}) with heavier molecules
- Shifts the wavelength



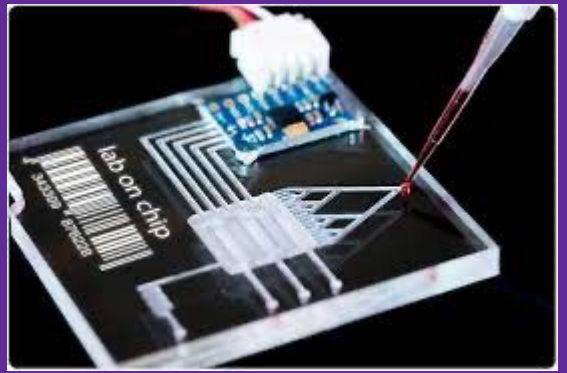
Measuring Ring Resonance

- Wavelengths that are trapped will resonate within the ring
- Trapped waves leave a negative peak in the measured spectrum of light leaving the ring
- As processed blood binds to the antigens on the ring the light wavelength sees a shift proportional to amount of bound material for each biomarker
- Shifts are detectable and correspond to biomarker concentrations



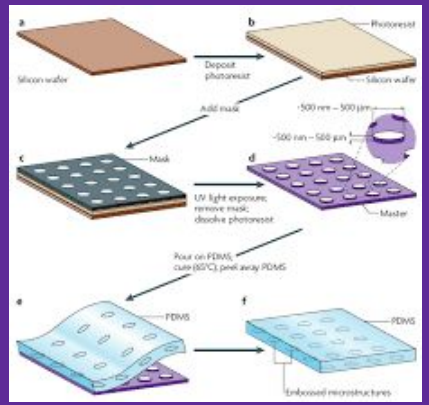
Proposal

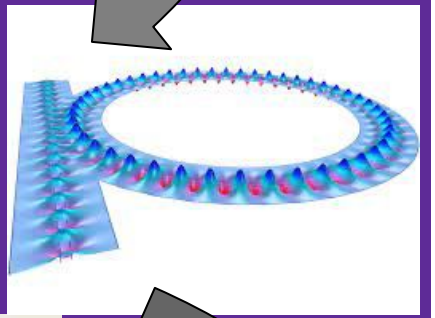
- Analyte → blood
- Immunoassay with multiplexed lab-on-a-chip approach in conjunction with ring resonance for analyte preparation and analysis
- Key TBI markers (S100B, GFAP, NF-L, UCH-L1, tau)
    - More/different biomarkers could be measured as well
    - Biomarkers are protective/neurotrophic signals released in response to TBI, many more than these five
- Blood→ plasma extraction→ optical ring resonance→ signal transducer→ concentration outputs for clinical use



Fabrication

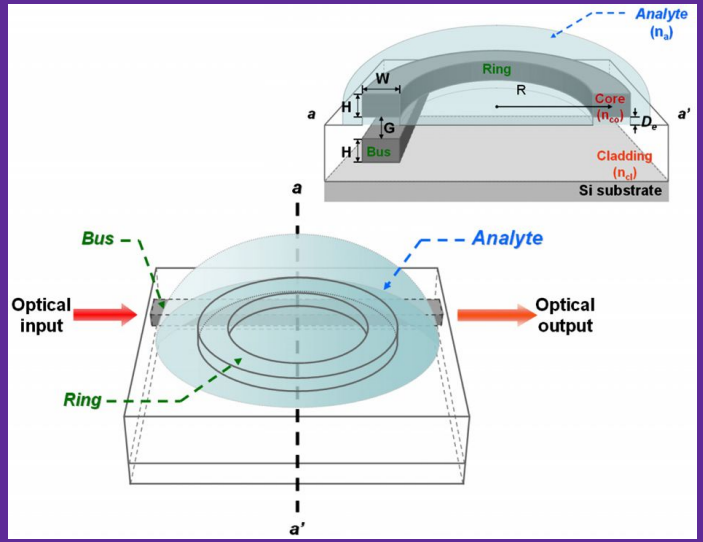
- Soft lithography to fabricate PDMS chip components with microchannels for blood flow
  - Photoresist, mask, UV exposure, PDMS casting
- Fabricate channels for blood separation (5) and subsequent integration of optical resonator
- Optical resonator is 4X6 mm with 48 rings organized into 12 clusters
  - Allows 1 sample → 12 assays
  - 2 samples → 6 assays each
- Design simple app (MATLAB) to stream data from resonator sensogram
  - Provide meaningful plots of relevant TBI markers for clinical interpretation

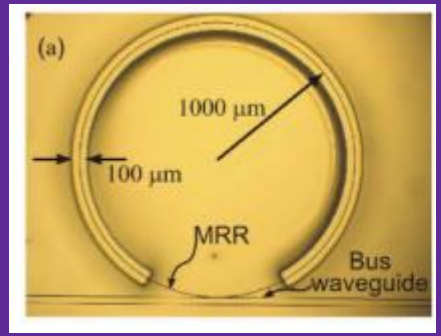




Detailed Optical Resonance Fabrication

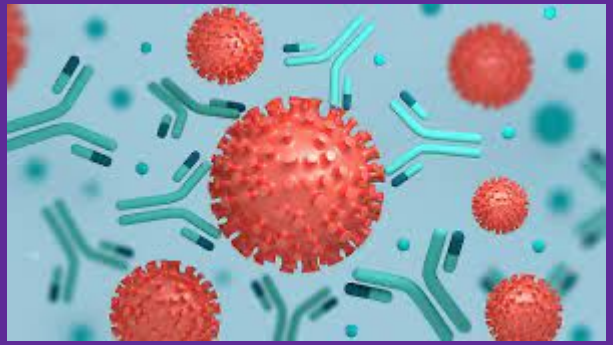
- Stereolithography to construct a PDMS chip with ring resonators and microfluidic channels
- Rings will 25 μm in diameter
- Chip will can be coated in per-fluoropolymer
- Rings will not be covered so material can flow over
- Free-space optical scanner & Grating coupler to be used for light in/out
- Antibodies will be deposited onto the silicon chips to correspond to GFAP, NF-L, UCH-L1, tau, and S100B
- Bus transducer will be used to measure signal changes

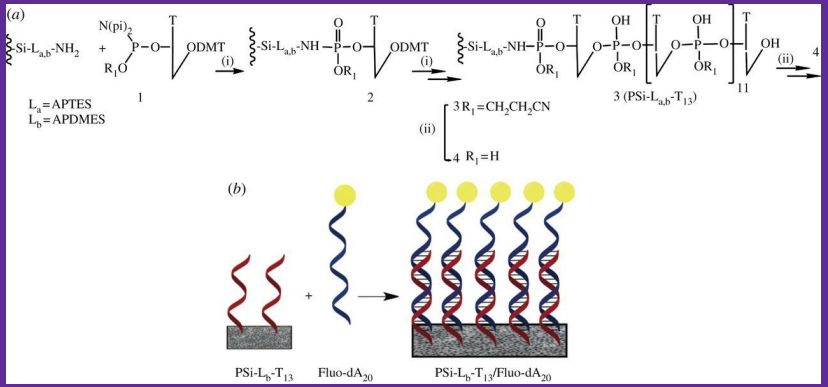




Antibody Spotting

- Rings can be washed with acetone and amino silane followed by water
- Rings will be dried with nitrogen gas
- An amino-amino crosslinker will be added
- Chips are then spotted with proper antibodies onto each ring
- Chips can then be dried, sealed, and stored at 2-8^0 C before use



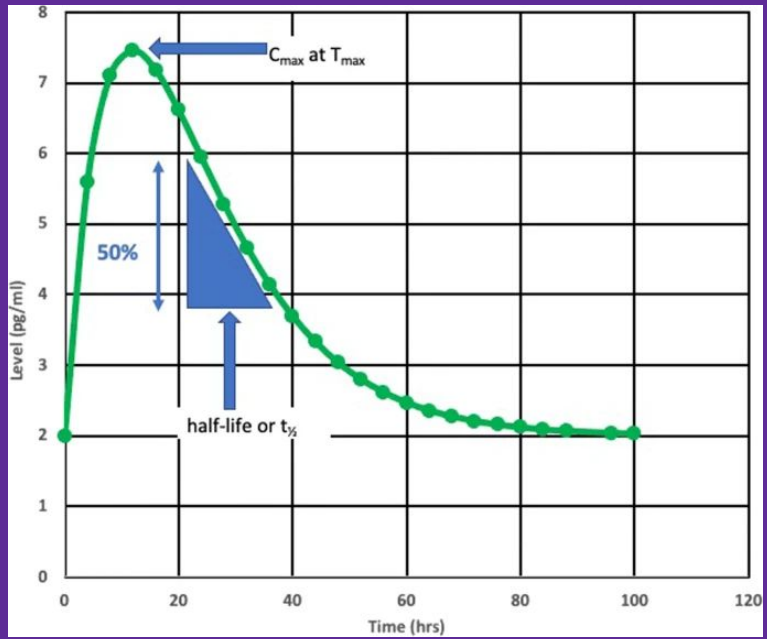


Testing

- Test with control samples, both positive and negative
- Compare results
  - Methods that use larger amounts of blood
  - Methods that use CSF
- Could use a population where TBI is common (Football, Hockey…)
- Test protein levels of athletes over certain time periods after event
- Use general population as a control group to see how they compare
- Start testing on animals if necessary
  - May not be necessary as testing isn't invasive
  - Just need a small blood sample

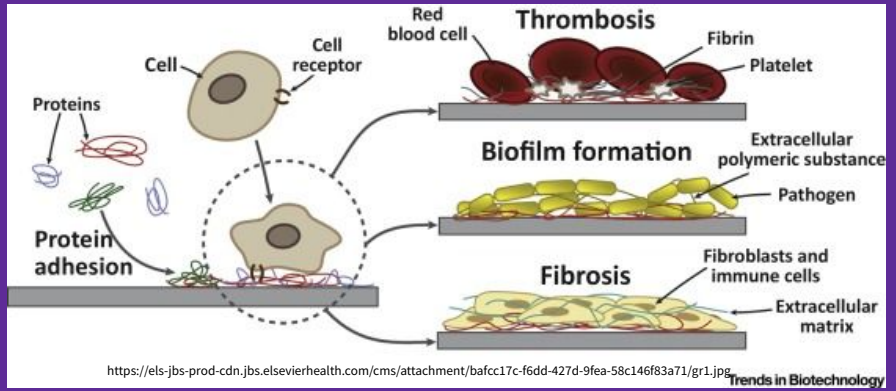
Limitations

- Less accurate than CSF measurements, but less traumatic
- Biomarkers can be elevated from trauma not including head injury
  - Especially that of S100b, a targeted biomarker
  - Would need a targeted range
- S100b has a half-life between 0.5-2hrs
  - Need samples rapidly, or focus on other proteins (GFAP)
- Need to determine better kinetic parameters of proteins



Biocompatibility

- Biocompatibility is a minor issue for our device as it is not meant to be implanted
- Anti-clotting materials would be important
  - Could use heparin as well
- Antifouling materials will help longevity of device and maintain accuracy of readings [10]



Summary

- Few accurate clinical predictors for TBI post injury
  - Results in many undiagnosed cases
  - Dangerous in case of re-injury as well as development
- Several Biomarkers that can indicate cell damage and death
  - Some are stronger indicators than others (S100b)
- Biochip can be created to obtain values for indicated Biomarkers
  - Optical Ring Resonance to perform multiplex immunoassays
  - Transducers to convert signal for reading
- Soft Lithography and Stereolithography used to fabricate device
  - Channels for Plasma separation
  - Antigens on chip corresponding to biomarkers
  - Bus Transducers for signal changes

References

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