

Red fluorescent protein : A powerful tool in deciphering spatial map of neurons.

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1. Summary

❑ Only colors of visible light that can cross the thick tissue barrier of the brain are between far red and red light. From this idea we devoted to inserting Red fluorescent protein gene in neurons that could respond to these lights.

❑ This could help us in locating specific neurons and their circuitry specific for an activity such as movement, thinking and behavioural change like depression, anxiety, emotions, arousal etc.

❑ We could be able to generate map of neurons by combining the information from RFP responses which was not possible by conventional imaging techniques such as fMRI and X-ray CT scan.

2. Introduction

❑ Previous attempt to map neurons used green fluorescent proteins which absorb blue light and emit green light but blue light get scattered and filtered while passing though thick brain tissue barrier. This limits getting fluroscence response from neurons that are located deep in the brain.

❑ One way to overcome this problem is cut brain into thin 2D slices to generate 2D images and finally combining the 2D image into a steriological map of neurons. So we miss out the actual data along the z axis and thus actual 3D map of neurons.

❑ One solution to this is the use of red fluorescent protein that requires far red light to fluorescence. Having longer wavelength red light is less scattered and crosses brain easily. Using RFP we can generate high resolution neural map.

3. How RFP works ?

Red fluorescent protein (RFP) is a fluorophore that fluoresces red-orange when excited. The original was isolated from Discosoma, and named **DsRed.** Improved variants of RFP include **mCherry, mOrange, mPlum, mRaspberry, mKO, TagRFP, mKate, mRuby.**

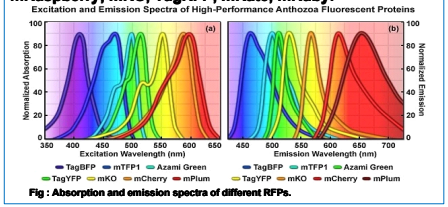


Fig : Absorption and emission spectra of different RFPs.

4. Material and Methods



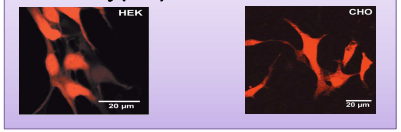
Fig : *Discosoma sp*



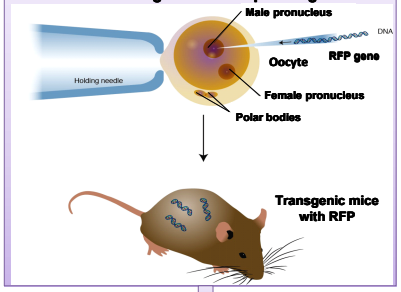
Fig : Heteractis crispa

RFP genes

Functional cytosolic expression of the various FPs, human embryonic kidney cells (HEK) and Chinese hamster ovary (CHO) cells



Generation of transgenic mice expressing RFP



Laser scanning of mice brain *and computational analysis of responses to generate neural map.*



Fig : Responses of RFP

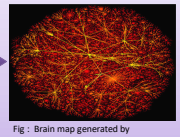
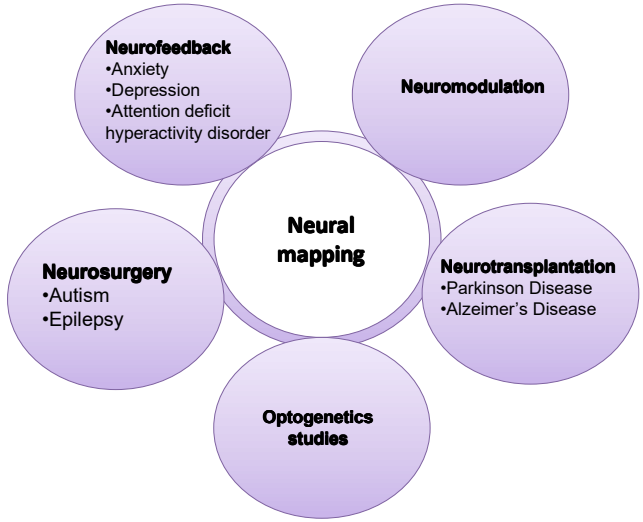


Fig : Brain map generated by computational analysis of images of neural networks.

5. Uses of brain mapping



6. Impact in Bangladesh

Making brain map using RFP will more efficient and powerful technology. As developed countries shift their interest to this new technology preexisting mapping techniques will become cheaper. Decrease in the cost of preexisting technology make it affordable in country like us. Brain mapping will help us treat autism and epilepsy in Bangladesh

7. Discussion

We have never seen a diagram of how all the neurons of the brain connect. A lot of our thinking is based on incomplete knowledge about brain of what is actually there. So we would like to see what is actually there. Neuromapping a pathway to discovery of intricate circuit in our brain.This may add new dimension to neurosurgery. Neuromodulation and neurofeedback of target neurons will be possible by knowing exact location of a particular neuron. Tracking light sensitive neurons will provide a good insight into **Optogenetics** studies.

8. Terminology

- **Neurotransplantation:** Replacement of dysfunctional neural cell with functional neuron
- **Neurofeedback :** A type of biofeedback that measures brain waves to produce a signal that can be used as feedback to teach self-regulation of brain function
- **Optogenetics:** Study that involve use of light to alter gene expression.
- **Neuromodulation :** Alteration of nerve activity through targeted delivery of a stimulus, such as electrical stimulation or chemical agents, to specific neurological sites in the body".

9. References

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