Imaging of 3D innervation zone (IZ) distribution in spastic muscles from high-density surface EMG recordings

NCT03302741

Version Date: 03/21/2017

Imaging of 3D innervation zone (IZ) distribution in spastic muscles from high-density surface EMG recordings

Protocol (CPHSHSC-MS-17-0174)

1. **Protocol Title: Imaging of 3D innervation zone distribution in spastic muscles from high-density surface EMG recordings**
   PI: Sheng Li, MD, PhD
   Co-Investigators: Ping Zhou, PhD, Yingchun Zhang, PhD (co-investigator from U of H)
   Study Coordinator: not identified
   Population: stroke subjects
   Number of Sites: single site
   Study Duration: planned for 2 years
   Subject Duration: estimated 24 subjects

2. **Specific aims and research questions:**

In this study, the goal of this project is to further evaluate the novel 3DIZI approach in post-stoke patients with muscle spasticity and to examine the feasibility of utilizing the 3DIZI approach to guide BTX injections in treating muscle spasticity.

3. **Background/literature review, Justification and Significance:**

The incidence of spasticity is reported to be 20%-40% in post-stroke survivors. It not only has downstream effects on the patients' quality of life, also lays substantial burden on the caregivers and the society. Botulinum neurotoxin (BTX) is considered as the first-line treatment for focal spasticity management. Intramuscular BTX injection has proven to be a relatively safe procedure (Bakheit 2006), but it may still cause side effects or even severe problems in patients such as blocking of autonomic nerves, muscle atrophy by anatomical denervation and immunological reactions (Klein 2002; Dressler and Hallett 2006; Warner et al. 2006; Simpson et al. 2008). The occurrence and severity of side effects are dependent on the delivered dose of toxin (Borodic et al. 1990; Eleopra et al. 1996), and it has been clinically shown that injection of a minimum effective dose reduces the presence of adverse effects (Brans et al. 1995). BTX acts on the neuromuscular junction (NMJ) and the effectiveness of the BTX injection depends on the proximity of the injection site to the NMJ (Jahn 2006). Studies have demonstrated that increasing the injection distance by 1cm from the NMJ indicated by the innervation zone (IZ) of muscles reduced the effect of BTX by 46% (Lapatki et al. 2011). Furthermore, IZ locations vary between muscles and individuals (Guzmán-Venegas et al. 2014). Therefore, it is critical to accurately identify the 3-dimensional (3D) IZ distributions in the spastic muscles for specific patients to guide BTX injections for the best clinical effect with the minimum dose.

Significant efforts have been taken to localize IZ locations from skin surfaces from multi-channel surface EMG recordings (Merletti et al. 2004; Barbero et al. 2011; Beretta Piccoli et al. 2014; Boccia and Rainoldi 2014; Guzmán-Venegas et al. 2014), however, there is no technique currently available for defining IZ distributions in 3D space of spastic muscles to guide BTX injections in specific patients. The minimal amplitude channel and/or the channel with phase reversal in the single differential signal have been utilized to identify the surface IZ locations (Merletti et al. 1999). The IZs can be localized over the skin

surface through visual inspections (Merletti et al. 2004; Barbero et al. 2011; Beretta Piccoli et al. 2014; Boccia and Rainoldi 2014; Guzmán-Venegas et al. 2014) or automatic algorithms such as bi-dimensional cross correlation (Cescon 2006; Beck et al. 2012; Cescon et al. 2014; Ullah et al. 2014), template matching (Mesin et al. 2009; Jahanmiri-Nezhad et al. 2015) or optical flow (Östlund et al. 2007). Those surface IZ mapping techniques have also been used to localize IZs over the skin surface to improve the BTX injection outcome, but the application of those surface localization method is limited by the lack of depth information of the IZs. Clinically, the motor point (MP) is used as the injection site of BTX since it is homologous to the IZ. However, a significant difference between the MP and IZ locations has been observed (Guzmán-Venegas et al. 2014). The bipolar high-density surface EMG mapping has also been used to localize IZs over the skin surface to improve the BTX injection outcome (Lapatki et al. 2011), but the application of this surface localization method is limited by the lack of the depth information of the IZs, particularly due to peripheral adaptive changes including atrophy and fat tissue deposit (Gracies 2005). 3D IZ location technique is therefore needed in order to improve clinical outcomes with minimum treatment dose and thus to minimize side effects.

Bioelectrical activity imaging approach has been well developed and successfully used to localize bioelectrical activities in the brain and the heart from scalp electroencephalogram (EEG) and body surface electrocardiogram (ECG) recordings, respectively (Mosher et al. 1993; Hämäläinen and Ilmoniemi 1994; Pascual-Marqui et al. 1994; Zhang et al. 2006; He et al. 2007; Zhang et al. 2008; Zhang et al. 2010). Similar to EEG and ECG signals, surface EMG signals are composed of the superimposed action potentials of many muscle fibers and are the summation of each motor unit action potentials (MUAPs). Several methods have been developed to localize internal muscle activities from multi-channel recordings of the surface EMG to improve its specificity to particular muscles. The bioelectrical activity imaging approach was first performed with an exhaustive search method based on a single sinusoidal current source model to localize internal muscle activities from surface EMG recordings (Jesinger and Stonick 1994). Latterly, a distributed tripole model was employed to model muscle activities and a generalized Tikhonov regularization approach was utilized to reconstruct internal muscle activities from surface EMG signals (Van Den Doel et al. 2008; Van Den Doel et al. 2011). We demonstrated the feasibility of imaging internal muscle activities from multi-channel intra-vaginal surface EMG measurements (Zhang et al. 2010), and recently developed a spatiotemporal muscle activity imaging approach to improve the reconstruction accuracy by incorporating the spatial and temporal components in the multi-channel surface EMG measurements (Wang et al. 2012). Those previous studies have demonstrated the feasibility of utilizing bioelectrical activity imaging methods to localize internal muscle activities from surface EMG recordings, but currently available muscle activity imaging approaches suffer from the low accuracy and specificity.

Composite surface EMG signals can be decomposed into their constituent MUAP trains (Ning et al. 2015). We recently developed a novel 3-dimentional innervation zone imaging approach (3DIZI) by combining the muscle activity imaging and surface EMG decomposition methods to image the distribution of IZs in the 3D space of the biceps from high-density surface EMG recordings (Liu et al. 2015). The surface location of the IZ of each decomposed motor unit (MU) was identified from the bipolar high-density MUAP trains, and was utilize to constrain the muscle activity imaging solution in the 3DIZI to improve its localization accuracy. Subsequently the 3DIZI can be used to accurately localize the IZs in the 3D space of target muscles. The 3DIZI has been validated in healthy the biceps in 2 male subjects, but has not been validated and applied to spastic muscles. In our recent study using surface linear-array EMG recordings (In Revision), we found IZ locations were asymmetrical between impaired and non-impaired biceps and large variations in IZ locations on the impaired side as well. The goal of this proposal is to validate the 3DIZI in spastic muscles, and to evaluate the feasibility of utilizing the 3DIZI to guide BTX injections in treating patients with muscle spasticity.

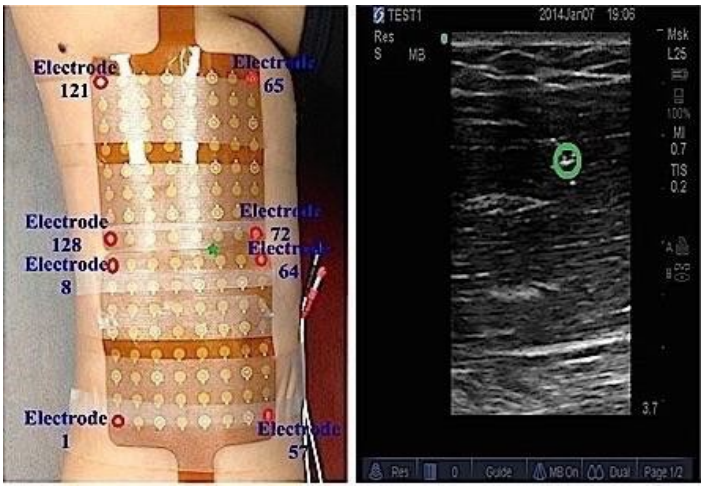
Innovation of this project

1) This research represents the first effort to image the 3D distributions of innervation zones in both healthy and spastic muscles. It provides ample opportunities for future basic research and clinical applications, such as the proposed application for guiding BTX injections.

2) This research will lead to a 3DIZ-guided BTX injection protocol which is innovative and clinically significant. Because BTX is injected to where its needs using this technique, the technique is able or expected to significantly reduce the dose, thus the cost of this very expensive treatment with improved clinical outcomes. Moreover, it is also expected to minimize possible adverse effects.

Preliminary results

In a pilot study (Liu et al. 2015), the 3DIZI was developed and evaluated with both simulated data and simultaneously recorded surface EMG and intramuscular EMG recordings from the biceps of 2 healthy subjects.

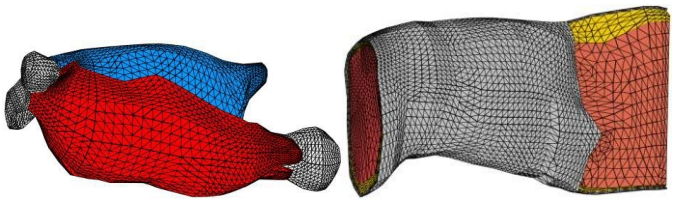


(a) (b)

Fig.1: shows (a) positions of 128-channel surface EMG recording electrodes (2 flexible 8 × 8 arrays with the electrode diameter of 4.5 mm, and the center-to-center electrode distance of 8.5 mm) and wire electrode (green star) over the subject's right upper arm, and (b) location of the inserted wire electrode (green circle) in the ultrasound image of the upper arm.

Development of the 3DIZI

Experimental setup and EMG recordings: Simultaneous surface EMG and intramuscular EMG measurements were acquired from the biceps of the two healthy male subjects with a 136-channel Refa (TMSi, Enschede, The Netherlands). The 128 unipolar channels were employed for surface EMG measurements and one bipolar channel was employed for simultaneous intramuscular EMG measurements for the validation purpose (Fig. 1(a)). A sampling rate of 2 KHz was adopted for both surface and intramuscular EMG recordings. The depth of the wire electrode inserted in the biceps was characterized (green circle) from the ultrasound images (Fig. 2(b)).



(a) (b)

Fig. 2: Computational model of (a) the bones and muscles, (b) the entire upper arm of the subject 1.

Computational model of the upper arm: A computational upper arm model was constructed from a general MR image data set and modified to match the ultrasound images of the subject's upper arm. The model consists of the skin, fat, biceps, triceps, compact bone and cancellous bone and was meshed with ANSYS 13.0 (ANSYS, Canonsburg, PA) into a finite element (FE) model for computation (Fig. 2). The entire FE model of the upper arm consists of 225,462 tetrahedron elements and 39,605 nodes. The conductivity values assigned to the skin, fat, muscle, compact bone and cancellous bone are 4.55×10^{-4} S/m, 0.0379 S/m, 0.2455 S/m, 0.02 S/m and 0.075 S/m, respectively (Lowery et al. 2002). An anisotropy ratio (longitudinal/transverse conductivities) of 5 was assumed for the biceps muscles in the model (Stoykov et al. 2005).

Surface EMG Decomposition: The K-means clustering and Convolution Kernel Compensation method (KmCKC) which we newly developed (Ning et al. 2015) was employed to decompose the 128-channel surface EMG signals into their constituent MUAP trains. The correlation between each decomposed MUAP train and the simultaneously recorded intramuscular EMG signals was calculated. A high correlation indicates that the corresponding MUAP train and the simultaneously recorded intramuscular EMG signals were generated by the same MU. The correlation calculation results showed that the 3rd and 6th MUAP trains in Subject 1 and the 1st and 5th MUAP trains in Subject 2 have the significantly high

correlation with the intramuscular EMG signals. The decomposed 128-channel surface EMG monopolar action potentials and the bipolar action potentials of the MU3 and MU6 in Subject 1 are presented in Fig. 3 as an example.

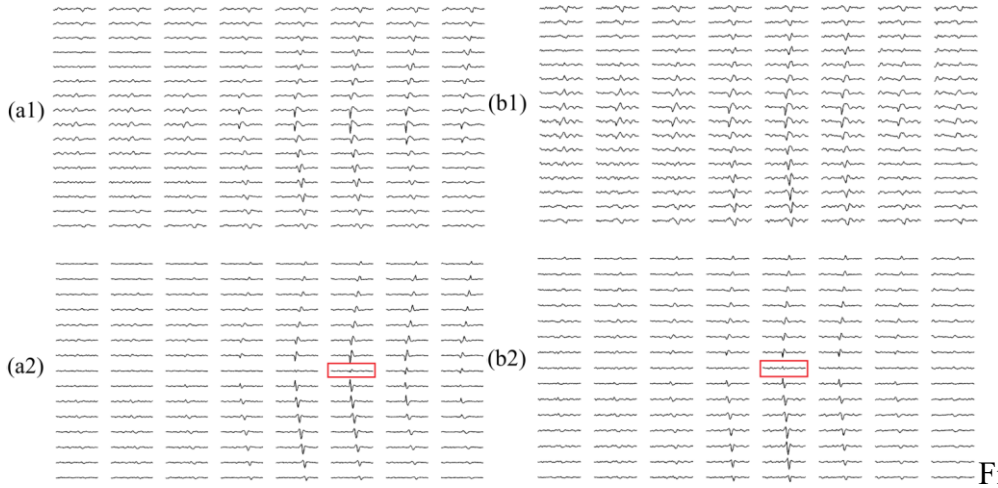


Fig. 3: (a1) and (a2) show monopolar and bipolar MUAPs of the MU3, and (b1) and (b2) show monopolar and bipolar MUAPs of the MU6, decomposed from 128-channel surface EMG recordings of the 1st subject's biceps. The red rectangles in (a2) and (b2) indicate the locations of IZs of the MU3 and MU6, which are very close to the 48th and 96th surface EMG electrode locations.

Source model: Considering the irregular and arbitrary shape of muscle fibers in human body, a distributed source model, which has the capability of accurately describing bioelectric activity without prior assumptions about its shape, was employed in the proposed 3DIZI (Zhang et al. 2006; Zhang et al. 2008; Zhang et al. 2010; Liu et al. 2015). Specifically a total number of 72,840 current dipoles were evenly distributed in the 3D space of the biceps with a spatial resolution of 2×2×2 mm^3, and each dipole has three orthogonal components that need to be determined.

3DIZI Inverse Solution: The linear relationship between the source space and measurement space is

$$L \cdot X = \Phi \quad (1)$$

where $\Phi=(\Phi_1, \Phi_2, \ldots, \Phi_M)^T$ is the vector of surface EMG measurements, $X=(x_1, x_2, \ldots, x_{3\times N})^T$ is the vector representing the strength of the distributed dipoles in the 3-dimential space of the biceps, $L = (L_1, L_2, \ldots, L_N)$ is the transfer matrix and L_i is an $M\times3$ matrix that represents the electric lead field of the i-th dipole. M refers to the number of surface recording electrodes over the skin and N refers to the number of dipole sources in the source space. The transfer matrix L can be calculated by solving the forward problem (Zhang et al. 2004). The weighted minimum-norm (WMN) regularization technique (Zhang et al. 2008; Zhang et al. 2010) was employed to solve the inverse problem and the electrical activities can be estimated as follow (Fuchs et al. 1999).

$$\hat{X} = (R^T W^T W R)^{-1} L^T (L (R^T W^T W R)^{-1} L^T + \lambda I)^{-1} \Phi \quad (2)$$

where W *is* a $3N\times3N$ weighted matrix which accounts for the undesired depth dependence, λ is the regularization parameter which is determined by means of the L-curve method (Hansen 1992), R is the source covariance matrix, which is constructed by taking the advantage of the surface location of the IZ identified from the corresponding bipolar MUAP trains, to constrain the muscle activity imaging solution in 3DIZI to improve its localization accuracy.

The surface position of the IZ of a particular MU can be identified from the bipolar map of the decomposed high-density MUAPs by checking the phases of the propagating signals. A cylinder with the diameter of 20 mm (Buchthal and Sten-Knudsen 1959; Merletti et al. 1999) is defined right underneath the identified surface IZ location and axially pointed to the normal direction of the skin surface, and the cylinder will create a cylindrical shaped sub-space in the target muscle space. A high weighting factor

(0.9) is applied to the cylindrical sub-space and a low weighting factor (0.1) is applied to the rest 3D muscle space, and the source covariance matrix R in Equation (2) is then calculated accordingly.

Evaluation of the 3DIZI in computer simulations

A series of computer simulations were conducted based on the FE model of the upper arm in Fig. 1 to evaluate and optimize the performance of the 3DIZI approach in localizing IZs in the 3D space of the biceps. One extended activation zone in an ellipsoidal shape with semi-axes length of 5 and 4 mm in parallel and normal to the muscle fiber directions were assumed in the biceps, with different depths (10, 15 and 20 mm depths from the skin surface) (Merletti et al. 1999; Merletti et al. 2008). At each depth, one activation zone was assumed at 6 locations in the biceps along the muscle fiber direction, or one pair of activation zones were assumed at 3 different locations which are symmetrical to the mid-axial plane the biceps. The electrical potentials at 128 EMG electrode locations were calculated based on the FE model as the simulated surface EMG measurements after adding Gaussian white noises at different noise levels (SNRs = ∞, 30, 25, 20) (Phinyomark et al. 2012).

The localization error (LE) was employed to evaluate the accuracy of the 3DIZI results. The LE is defined as the spatial distance between the center of the assumed target activation zone and that of the reconstructed muscle activation zone. For the cases with two activation zones, the LE was calculated by averaging the two individual LEs. Fig. 4 presents the mean and standard deviation of LEs achieved at different noise levels and source depths. The noise and source depth does affect the accuracy of the 3DIZI results, but the all mean LEs are less than 5 mm for all the cases, which demonstrates the high performance of the 3DIZI.

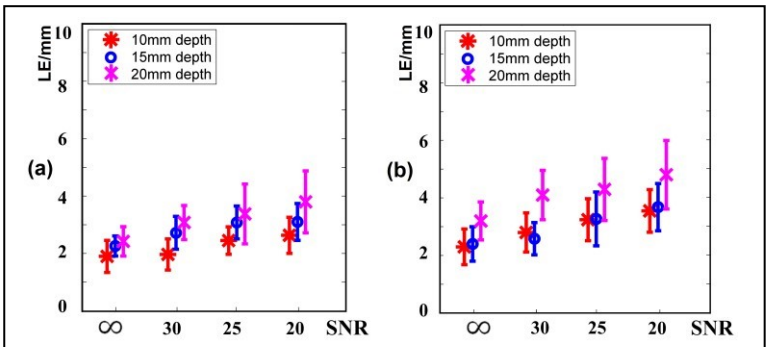


Fig. 4: Means and standard deviations of LEs for (a) one activation zone and (b) two activation zones with 4 noise levels and three source depths.

Evaluation of the 3DIZI approach in healthy subjects

The correlation results in the model development study show that the MU1 and MU5 in Subject 1 were simultaneously measured by the inserted wire electrode and surface EMG electrodes. Fig. 1(a) and Fig. 3 also show that the wire electrode was inserted into the IZs for the MU1 and MU5. Those observations suggest that the muscle activities associated with the two MUs were successfully recorded by both the surface EMG and intramuscular wire electrode at the time instant, when the MUAP trains were just initiated in their IZs. Therefore the true IZ location of the MU1 and MU5 in Subject 1 is indicated by the location of the inserted wire electrode which was characterized from the ultrasound images in Fig. 1(b). The 3DIZI was utilized to image the IZ locations of the MU1 and MU5 in Subject 1 from their MUAPs, as shown in Fig. 5 (a1) - (c1). It was found the two reconstructed IZs and their overlap fully covered the insertion location of the wire electrode. The wire electrode was not inserted into any IZs of the biceps of the subject 2, but very similarly, the propagating activities of the MU3 and MU6 were both successfully measured by the surface EMG and intramuscular EMG electrodes. The reconstructed muscle activities in the propagating activation zones are presented in Fig. 5 (a2) - (c2). The overlap of the propagating activation zones of the two MUs in Subject 2 fully covers the location of the inserted wire electrode. The pilot results demonstrate the high accuracy of the 3DIZI in localizing the IZs from high-density surface EMG recordings, given the fact that fine wire electrodes used in this study can only detect activities in a

very small muscle volume directly surrounding the electrode tip (Basmajian and De Luca 1985; Merletti et al. 2004).

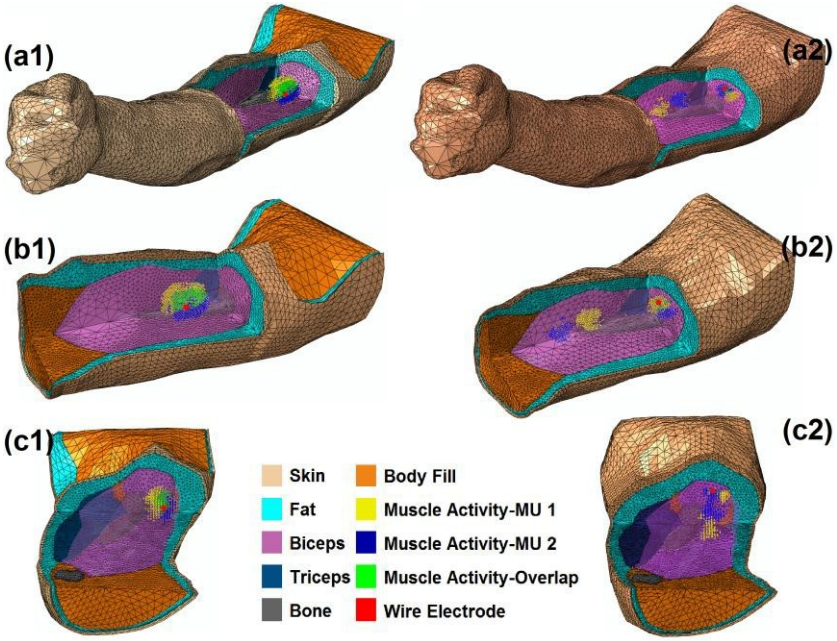


Fig. 5: (a1), (b1) and (c1) show the overview, lateral view and axial veiw of the reconstructed invervation zones of the two MUs and their overlap in Subect 1, respectively. (a2), (b2) and (c2) show the overview, lateral view and axial veiw of the reconstructed invervation zones of the two MUs in Subect 2. Blue and yellow dots represent the invervation zone of the two MUs respectively in each subject, green dots represent their overlap area. The two red dots represent the location of the inserted wire electrode.

Therefore, this research study will evaluate the performance of the 3DIZI in imaging the 3D distributions of IZ zones in spastic muscles (Experiment 1), and examine the feasibility of utilizing the 3DIZI to guide BTX injections in treating patients with post-stoke spasticity (Experiment 2).

4. Research design and methods:

Location: Neurorehabilitation Research Laboratory at TIRR Memorial Hermann Hospital

Experiment 1: Evaluation of the performance of the 3DIZI in spastic muscles

The purpose is to evaluate the performance of the 3DIZI approach in localizing IZs in the 3D space of the spastic muscles with simultaneous high-density surface EMG and intramuscular EMG recordings.

Eight patients with spastic biceps will be recruited to participate in this validation study in TIRR. The same protocol will be used as described in our preliminary study (Liu et al. 2015).

Simultaneous surface EMG and intramuscular EMG recordings: Simultaneous surface EMG and intramuscular EMG measurements will be acquired from the spastic biceps of the patients with a 136-channel Refa (TMSi, Enschede, The Netherlands). Patients will be seated comfortably on a height-adjustable chair. The arm to be tested will be secured firmly on a customized apparatus with the elbow joint at approximately 90 of flexion and the shoulder at approximately 45 of abduction and 30 of flexion. The 128-channel unipolar surface EMG signals will be recorded with 2 flexible 2-dimensional 64-channel (in 8x8 formation, individual recording probe 4.5 mm in diameter, center-to-center probe distance 8.5

mm) surface electrode array (TMSi, Enschede, The Netherlands). The skin of the tested muscle will be carefully prepared, and the electrode array was attached to the biceps muscles with a double adhesive sticker and further secured with medical tapes. A coating fine wire electrode (VIASYS Healthcare, Madison, WI) will be inserted into the mid-axial section of the biceps to record bipolar intramuscular EMG signals. Ultrasound scan (M Turbo, SonoSite, Bothell, WA) will be performed on the biceps to identify the location of the inserted wire electrode. Patients will be asked to contract their impaired biceps to perform maximum voluntary contraction (MVC) of elbow flexion against the vertical plates 3 times. Each trial will last for 10 sec. A sampling rate of 2 KHz will be used for simultaneous surface and intramuscular EMG recordings during muscle contraction.

Validation: 1) The recorded 128-channel surface EMG signals will be first decomposed into their constituent MUAP trains using the KmCKC approach (Ning et al. 2015); 2) Correlations between each MUAP trains and the intramuscular EMG signals will be calculated to identify the MUs which were simultaneously recorded by both the surface EMG and wire EMG electrodes; 3) the muscle activities of the MUs, which were simultaneously recorded by both surface EMG and wire EMG electrodes, will be reconstructed in the 3D space of the biceps from their MUAPs respectively using the 3DIZI approach; 4) the localization error (LE), defined as the distance between the center of the reconstructed muscle activation zone or IZs and the location of the inserted wire electrode identified in ultrasound images, will be calculated. The mean value and standard deviation of LEs achieved from each patient will be calculated to evaluate the accuracy of the 3DIZI results.

Pitfalls: Attempts will be made to insert the wire electrode into IZs in the mid-axial section of the biceps so that muscle activities in IZs can be recorded. However, it is possible that the wire electrode will not be inserted into any IZ of the specific biceps and no muscle activities in IZs can be measured. In this case, the propagating muscle activities measured by the wire electrode, as demonstrated in Fig. 5(a2) - (c2) will be used to evaluate the proposed 3DIZI approach. For patients who cannot contract their spastic biceps efficiently, electrical stimulation will be delivered to the musculocutaneous nerve using an electrical stimulator (D7SA, Digitimer Ltd, Hertfordshire, England) to generate a maximum response. Techniques for removal of stimulation artifacts have been recently developed by our group (Li et al. 2014).

Experiment 2: Evaluation of the feasibility of utilizing the 3DIZI to optimize the BTX injection

The purpose of this Aim is to evaluate the feasibility of utilizing the 3DIZI technique to optimize BTX injections in patients with focal spasticity. Clinical and quantitative assessments of spastic biceps of stroke subjects between 3DIZI-guided and standard injections at baseline, 3 weeks, and 3 months post-injections will be compared. The clinical effect of BTX injection usually reaches to its peak at about 3 weeks, and lasts for 3 months. It is expected that outcomes (clinical and quantitative measures) will be better in the 3DIZI-guided injection group than in the standard injection group at 3 weeks and 3 months post-injection. Both groups are expected to have comparable baseline measures.

Patient selection: We plan to recruit 16 post-stroke subjects who are currently receiving BTX injections for their spastic biceps every 3 months at TIRR Outpatient Spasticity Management Clinic into two groups: the IZ-guided injection group and the standard injection group (Please see human subject protect section for characteristics of stroke patients).

BTX injections: Both groups of patients will be recruited prior to their routine BTX injection schedule. Baseline assessment (screening, clinical assessment, and quantitative assessment) will be performed 1 day prior to injection. Stroke patients with elbow flexors spasticity of modified Ashworth scale (MAS) of 2 or 3 will be recruited. The number of MAS (2s or 3s) will be matched between two groups. The type of BTX (we will choose botox because it is most commonly used in US) and amount of BTX (usually 100 units) and dilutions (usually double dilution) will be consistent between two groups. These parameters (type, dose, dilution) are standard of care at TIRR Spasticity Management Clinic. For standard injection procedures, target muscles will be visualized under ultrasound imaging which is operated by an experienced and dedicated technician. Position of needle tip within the target muscle is visualized prior to

injection. Ultrasound guidance can help ensure depth of needle tip location, i.e., to make sure the needle tip is within the muscle, but it is not able to tell where it is located with reference to the IZs of the entire muscle. However, in the IZ-guided injection technique, IZ location obtained using the 3DIZ will be first marked over the skin surface of the muscle and the depth of the IZ will be also provided. The 3DIZ as described in Aim 1 will be applied to the IZ-guided injection group 1 day prior to scheduled injection. The surface location and depth information of the IZ will be used to guide where the needle tip needs to go. Currently, patients commonly receive 1 to 2 injection sites, occasionally 3 sites for biceps muscles. To standardize the procedure, we will choose 2 sites for all patients.

Treatment outcome evaluation and comparison: Treatment outcomes include clinical assessment of spasticity (MAS scale, see Human Subject Protection section) and quantitative assessment. Standard physical therapy will be ordered to both groups as part of standard of care for patients after BTX injections to maximize the outcomes. For quantitative assessment, we will adopt the same protocol in our recent study which quantified elbow flexors spasticity by measuring reflex torque (Figure 6)(Bhadane et al. 2015).

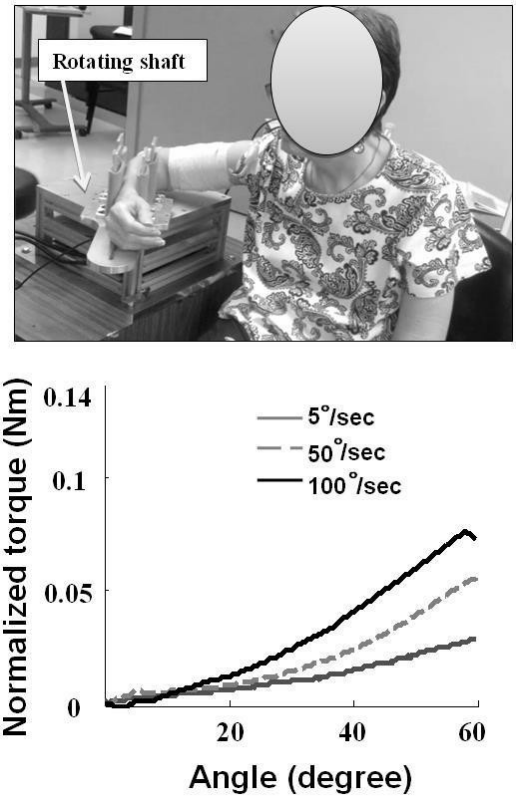


Fig. 6: Experimental setting (upper) and representative data (lower).

Each subject will receive a total of 60 degrees of computer-controlled elbow extension stretching at different speeds. The stretch ends at 10 degrees beyond the resting angle of the elbow joint during standing to offset the baseline difference among subjects (Bhadane et al. 2015). From the angle-torque relations, reflex torque is obtained after subtracting passive torque at 5˚/sec from those at 50˚/sec or 100˚/sec. reflex torque is considered to reflect neural component of muscle spasticity (Kamper et al. 2001; Kamper et al. 2003; Li et al. 2006). It is expected to be reduced with spasticity reduction after neuromuscular blockade by Botox.

These measures will be made at baseline, 3 weeks and 3 months post-injection. Two-way ANOVA with factors of GROUP (x2) and TIME (x3) will be performed on reflex torque values. It is anticipated that MAS scores, reflex torque values are comparable at baseline, but will be significantly reduced at 3 weeks and 3 months for both groups. Reduction is expected to be significantly more in the IZ-guided injection group than in the standard group. We do anticipate great reduction in elbow flexor spasticity as quantified by reflex-torque measurement after the IZ-guided injection. However, MAS scores are not as reliable in general (Craven and Morris 2010), and could be influenced by other elbow flexors.

Pitfalls: If other elbow flexors are required to be injected but may not be consistent across subjects, it is unethical not to inject (treat) these muscles. Assessment of biceps spasticity may be influenced and thus MAS scores may not accurately reflect the spasticity reduction effect from biceps muscles alone. Efforts will be made to balance treatment plans between groups in the recruitment process.

Limitation: Eight patients and 16 patients (8 patients per group) are proposed for Exp 1 and Exp 2 respectively. We expect that 8 subjects in each group should be sufficient to detect difference should it exist, according a recent study (Lapatki et al. 2011). These estimated numbers fit in the scope of work of this 2-year exploratory project. A power analysis will be performed at the completion of this project to decide whether or how many more subjects will be needed for a future study. Full-time biostatistians are available at both UH and TIRR (see Facilities and Other Resources section) for statistical analysis support.

The implementation of the 3DIZI technique currently proposed in the proposal is relatively complicated, because the main purpose of this project is to evaluate the performance of the proposed 3DIZI and examine the feasibility of its application to clinic. As the next step, we plan to simplify the implementation of the 3DIZI technique to make it appropriate for clinic use.

Specimens:
No specimens will be collected

5. Human Subjects

As mentioned above, it is a limitation in estimation of the number of subjects for each experiment. Eight patients and 16 patients (8 patients per group) are proposed for Exp 1 and Exp 2 respectively. We expect that 8 subjects in each group should be sufficient to detect difference should it exist, according a recent study (Lapatki et al. 2011). These estimated numbers fit in the scope of work of this 2-year exploratory project. A power analysis will be performed at the completion of this project to decide whether or how many more subjects will be needed for a future study. Full-time biostatistians are available at both UH and TIRR (see Facilities and Other Resources section) for statistical analysis support.

Gender will be balanced

Inclusion and Exclusion Criteria:

Inclusion criteria for participation in the study will include:
- a history of not more than one stroke which occurred at least 6 months prior to study enrollment;
- elbow flexor spasticity rated at 2 or 3 on Modified Ashworth scale (MAS);
- receiving repeated botulinum toxin injection every 3-4 months;
- absence of excessive pain in the paretic upper limb;
- capacity to provide informed consent, with Mini-Mental State Examination (MMSE) must be 25 or higher;

The following modified Ashworth scale (MAS) will be used for spasticity assessment:
- 0 -No increase in muscle tone;
- 1 -Slight increase in muscle tone, manifested by a catch and release or by minimal resistance at theend of the range of motion when the affected part(s) is moved in flexion or extension;
- 1+ -Slight increase in muscle tone, manifested by a catch, followed by minimal resistance throughout
- the remainder (less than half) of the ROM;
- 2 -More marked increase in muscle tone through most of the ROM, but affected part(s) easily moved;
- 3 -Considerable increase in muscle tone, passive movement difficult;
- 4 -Affected part(s) rigid in flexion or extension.

Main exclusion criteria:
- recent botulinum toxin injection < 4 months;
- recent changes in antispastic medications <3 weeks (i.e., the antispastic medication regime is not
- stable;
- Changes in antispastic medications (such as baclofen, tizanidine, dantrolene etc) during the followup research visits. (NOTE: it is clinically rare for patients who receive repeated injections to change their antispastic medications);
- history of spinal cord injury or traumatic brain damage;
- history of serious medical illness such as cardiovascular or pulmonary complications;

- any condition that, in the judgment of a physician, would prevent the person from participating. Efforts will be made to match a variety of measures between two groups, such as age, gender, time from stroke, spasticity in other elbow flexors (e.g., brachioradialis), and injection sites. Given the volume of patients in TIRR Spasticity Management Clinic, we are confident that we can achieve this goal.

Potential subject populations and recruitment: Subjects will be recruited from TIRR outpatient clinic.

6. Data Collection and Analysis

A desktop computer will be used for data acquisition and processing. Data will be saved for off-line analysis using customized MatLab software and Statistica software.

Experiment 1: This is a validation study. Detailed information has been provided above.

Experiment 2: These measures will be made at baseline, 3 weeks and 3 months post-injection. Two-way ANOVA with factors of GROUP (x2) and TIME (x3) will be performed on reflex torque values. It is anticipated that MAS scores, reflex torque values are comparable at baseline, but will be significantly reduced at 3 weeks and 3 months for both groups. Reduction is expected to be significantly more in the IZ-guided injection group than in the standard group. We do anticipate great reduction in elbow flexor spasticity as quantified by reflex-torque measurement after the IZ-guided injection. However, MAS scores are not as reliable in general (Craven and Morris 2010), and could be influenced by other elbow flexors.

Demographic data will be analyzed descriptively. Personal information will be de-identified.

7. Potential Risks/Discomforts:

There are very few risks associated with the proposed studies. The self-adhesive surface electrodes used to record surface EMG may produce minor irritation (redness, itching) of the skin under the electrodes. There are minimum risks associated with needle EMG. Subject may feel irritation, discomfort and pain during needle insertion. Needle insertion may also associate with bleeding. Bleeding is minimum should it occur. Electrical stimulation may cause an unusual sensation that can be painful at higher intensities. Note that risks associated with botulinum toxin injections will be addressed by treating physicians clinically, not in the scope of this research project. Post-Botox injection pain with 3DIZI guidance or conventional guidance is rare. It is common clinical practice that patients are advised to take over the counter pain killer for such pain if it does occur.

8. Benefits:

The benefit of this research will be the contribution it makes to knowledge, particularly our understanding of motor unit 3D localization within the spastic-paretic muscles, and development of possible guidance for botulinum toxin injection. Stroke subjects who receive 3D IZ guided injection may benefit from the research project in that the effect of injection may be maximized.

9. Risk-benefit Ratio:

In view of the minimal risk, the knowledge to be gained far outweighs the risks.

10. Consent Procedures:

Informed consent will be obtained from the subject. After the subject is identified and is interested in participating, informed, written consent will be obtained by the investigator.

11. Confidentiality Procedures:

In order to minimize risk to confidentiality, all data will be de-identified, coded with a study-specific identification number, maintained on a password-protected server, and/or kept in a locked office. No findings will be released without written authorization by the subject or requests by law.

12. Costs:

The subject will not be expected to pay any costs. Routine BoNT injections are part of their standard of care

13. Payments:

There will be no costs to subjects. Instead, subjects will receive a gift card of $40 for their participation in Experiment 1. They will receive gift cards in a total amount of $80 after last visit (a total of 3 visits) in Exp 2.

14. References:

Bakheit, A. M. O. (2006) The possible adverse effects of intramuscular botulinum toxin injections and their management. *Current drug safety* 1,271-279

Barbero, M., Gatti, R., Conte, L. L., Macmillan, F., Coutts, F., Merletti, R. (2011) Reliability of surface EMG matrix in locating the innervation zone of upper trapezius muscle. *Journal of Electromyography and Kinesiology* 21,827-833

Basmajian, J. V., De Luca, C. (1985) Muscles alive. *Proceedings of The Royal Society of Medicine* 278,126

Beck, T. W., DeFreitas, J. M., Stock, M. S. (2012) Accuracy of three different techniques for automatically estimating innervation zone location. *Computer methods and programs in biomedicine* 105,13-21

Beretta Piccoli, M., Rainoldi, A., Heitz, C., et al. (2014) Innervation zone locations in 43 superficial muscles: toward a standardization of electrode positioning. *Muscle & nerve* 49,413-421

Bhadane, M. Y., Gao, F., Francisco, G. E., Zhou, P., Li, S. (2015) Correlation of resting elbow angle with spasticity in chronic stroke survivors. *Front. Neurol.* 6,183

Boccia, G., Rainoldi, A. (2014) Innervation zones location and optimal electrodes position of obliquus internus and obliquus externus abdominis muscles. *Journal of Electromyography and Kinesiology* 24,25-30

Borodic, G. E., Joseph, M., Fay, L., Cozzolino, D., Ferrante, R. J. (1990) Botulinum A toxin for the treatment of spasmodic torticollis: dysphagia and regional toxin spread. *Head & neck* 12,392-399

Brans, J., De Boer, I., Aramideh, M., Speelman, J., de Visser, B. O. (1995) Botulinum toxin in cervical dystonia: low dosage with electromyographic guidance. *Journal of neurology* 242,529-534

Buchthal, F., Sten-Knudsen, O. (1959) Impulse propagation in striated muscle fibers and the role of the internal currents in activation. *Annals of the New York Academy of Sciences* 81,422-445

Cescon, C. (2006) Automatic location of muscle innervation zones from multi-channel surface EMG signals. In: Medical Measurement and Applications, 2006. MeMea 2006. IEEE International Workshop on. IEEE, pp 87-90

Cescon, C., Riva, D., Začesta, V., et al. (2014) Effect of vaginal delivery on the external anal sphincter muscle innervation pattern evaluated by multichannel surface EMG: results of the multicentre study TASI-2. *International urogynecology journal* 25,1491-1499

Craven, B., Morris, A. (2010) Modified Ashworth scale reliability for measurement of lower extremity spasticity among patients with SCI. *Spinal Cord* 48,207-213

Dressler, D., Hallett, M. (2006) Immunological aspects of Botox®, Dysport® and MyoblocTM/NeuroBloc®. *European Journal of Neurology* 13,11-15

Eleopra, R., Tugnoli, V., Caniatti, L., De Grandis, D. (1996) Botulinum toxin treatment in the facial muscles of humans Evidence of an action in untreated near muscles by peripheral local diffusion. *Neurology* 46,1158-1160

Fuchs, M., Wagner, M., Köhler, T., Wischmann, H.-A. (1999) Linear and nonlinear current density reconstructions. *Journal of clinical Neurophysiology* 16,267-295

Gracies, J. M. (2005) Pathophysiology of spastic paresis. I: Paresis and soft tissue changes. *Muscle & nerve* 31,535-551

Guzmán-Venegas, R. A., Araneda, O. F., Silvestre, R. A. (2014) Differences between motor point and innervation zone locations in the biceps brachii. An exploratory consideration for the treatment of spasticity with botulinum toxin. *Journal of Electromyography and Kinesiology* 24,923-927

Hämäläinen, M. S., Ilmoniemi, R. (1994) Interpreting magnetic fields of the brain: minimum norm estimates. *Medical & biological engineering & computing* 32,35-42
Hansen, P. C. (1992) Analysis of discrete ill-posed problems by means of the L-curve. *SIAM review* 34,561-580
He, B., Liu, C., Zhang, Y. (2007) Three-dimensional cardiac electrical imaging from intracavity recordings. *Biomedical Engineering, IEEE Transactions on* 54,1454-1460
Jahanmiri-Nezhad, F., Barkhaus, P. E., Rymer, W. Z., Zhou, P. (2015) Innervation zones of fasciculating motor units: observations by a linear electrode array. *Frontiers in Human Neuroscience* 9
Jahn, R. (2006) A neuronal receptor for botulinum toxin. *Science* 312,540-541
Jesinger, R. A., Stonick, V. L. (1994) Processing signals from surface electrode arrays for noninvasive 3D mapping of muscle activity. In: Digital Signal Processing Workshop, 1994., 1994 Sixth IEEE. IEEE, pp 57-60
Kamper, D., Harvey, R., Suresh, S., Rymer, W. (2003) Relative contributions of neural mechanisms versus muscle mechanics in promoting finger extension deficits following stroke. *Muscle & nerve* 28,309-318
Kamper, D., Schmit, B., Rymer, W. (2001) Effect of muscle biomechanics on the quantification of spasticity. *Annals of biomedical engineering* 29,1122-1134
Klein, A. W. (2002) Complications and adverse reactions with the use of botulinum toxin. *Disease-A-Month* 48,336-356
Lapatki, B., Van Dijk, J., Van de Warrenburg, B., Zwarts, M. (2011) Botulinum toxin has an increased effect when targeted toward the muscle's endplate zone: A high-density surface EMG guided study. *Clinical Neurophysiology* 122,1611-1616
Li, S., Kamper, D. G., Rymer, W. Z. (2006) Effects of changing wrist positions on finger flexor hypertonia in stroke survivors. *Muscle & nerve* 33,183-190
Li, S., Liu, J., Bhadane, M., Zhou, P., Rymer, W. Z. (2014) Activation deficit correlates with weakness in chronic stroke: Evidence from evoked and voluntary EMG recordings. *Clinical Neurophysiology* 125,2413-2417
Liu, Y., Ning, Y., Li, S., Zhou, P., Rymer, W. Z., Zhang, Y. (2015) Three-Dimensional Innervation Zone Imaging from Multi-Channel Surface EMG Recordings. *International Journal of Neural Systems* 25,1550024
Lowery, M. M., Stoykov, N. S., Taflove, A., Kuiken, T. (2002) A multiple-layer finite-element model of the surface EMG signal. *Biomedical Engineering, IEEE Transactions on* 49,446-454
Merletti, R., Bottin, A., Cescon, C., et al. (2004) Multichannel surface EMG for the non-invasive assessment of the anal sphincter muscle. *Digestion* 69,112-122
Merletti, R., Conte, L. L., Avignone, E., Guglielminotti, P. (1999) Modeling of surface myoelectric signals. I. Model implementation. *Biomedical Engineering, IEEE Transactions on* 46,810-820
Merletti, R., Holobar, A., Farina, D. (2008) Analysis of motor units with high-density surface electromyography. *Journal of electromyography and kinesiology* 18,879-890
Mesin, L., Gazzoni, M., Merletti, R. (2009) Automatic localisation of innervation zones: a simulation study of the external anal sphincter. *Journal of Electromyography and Kinesiology* 19,e413-e421
Mosher, J. C., Spencer, M. E., Leahy, R. M., Lewis, P. S. (1993) Error bounds for EEG and MEG dipole source localization. *Electroencephalography and clinical Neurophysiology* 86,303-321
Ning, Y., Zhu, X., Zhu, S., Zhang, Y. (2015) Surface EMG Decomposition Based on K-means Clustering and Convolution Kernel Compensation. *Biomedical and Health Informatics, IEEE Journal of* 19,471-477
Östlund, N., Gerdle, B., Karlsson, J. S. (2007) Location of innervation zone determined with multichannel surface electromyography using an optical flow technique. *Journal of Electromyography and Kinesiology* 17,549-555
Pascual-Marqui, R. D., Michel, C. M., Lehmann, D. (1994) Low resolution electromagnetic tomography: a new method for localizing electrical activity in the brain. *International Journal of psychophysiology* 18,49-65
Phinyomark, A., Phukpattaranont, P., Limsakul, C. (2012) Fractal analysis features for weak and single-channel upper-limb EMG signals. *Expert Systems with Applications* 39,11156-11163
Simpson, D., Gracies, J., Graham, H., et al. (2008) Assessment: Botulinum neurotoxin for the treatment of spasticity (an evidence-based review) Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology* 70,1691-1698
Stoykov, N. S., Lowery, M. M., Kuiken, T. A. (2005) A finite-element analysis of the effect of muscle insulation and shielding on the surface EMG signal. *IEEE transactions on biomedical engineering* 52,117-121
Ullah, K., Cescon, C., Afsharipour, B., Merletti, R. (2014) Automatic detection of motor unit innervation zones of the external anal sphincter by multichannel surface EMG. *Journal of Electromyography and Kinesiology* 24,860-867
Van Den Doel, K., Ascher, U. M., Pai, D. K. (2008) Computed myography: three-dimensional reconstruction of motor functions from surface EMG data. *Inverse Problems* 24,065010
Van Den Doel, K., Ascher, U. M., Pai, D. K. (2011) Source localization in electromyography using the inverse potential problem. *Inverse Problems* 27,025008

Wang, J., Zhang, Y., Zhu, X., Zhou, P., Liu, C., Rymer, W. Z. (2012) A novel spatiotemporal muscle activity imaging approach based on the Extended Kalman Filter. In: Engineering in Medicine and Biology Society (EMBC), 2012 Annual International Conference of the IEEE. IEEE, pp 6236-6238

Warner, S. E., Sanford, D. A., Becker, B. A., Bain, S. D., Srinivasan, S., Gross, T. S. (2006) Botox induced muscle paralysis rapidly degrades bone. *Bone* 38,257-264

Zhang, Y., Ding, L., van Drongelen, W., Hecox, K., Frim, D. M., He, B. (2006) A cortical potential imaging study from simultaneous extra-and intracranial electrical recordings by means of the finite element method. *NeuroImage* 31,1513-1524

Zhang, Y., van Drongelen, W., Kohrman, M., He, B. (2008) Three-dimensional brain current source reconstruction from intra-cranial ECoG recordings. *NeuroImage* 42,683-695

Zhang, Y., Wang, D., Timm, G. W. (2010) A three-dimensional muscle activity imaging technique for assessing pelvic muscle function. *Inverse Problems* 26,115018

Zhang, Y., Zhu, S., He, B. (2004) A second-order finite element algorithm for solving the three-dimensional EEG forward problem. *Physics in Medicine and Biology* 49,2975